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RELATION OF SULPHATES TO PLANT GROWTH AND COMPOSITION

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HISTORICAL REVIEW

The oxidation of sulphur in the soil and the relation of the products formed is plant growth, bacterial development and activity, and to the release of other forms of plant food have been reported upon by many investigators. In reviewing the work, many of the writers have reported beneficial results from the use of sulphur fertilizers, especially with those plants high in protein and other sulphur-containing compounds. Various views are given as to how sulphur functions in producing these increased yields. Analysis of soils reported by Hart and Peterson (*11*),[1] Shedd (*22*, *23*), Brown and Kellogg (*7*), and Swanson and Miller (*26*), show a lower sulphur content in the cultivated soil as compared to the phosphorus, while many of the cultivated plants show a larger content of sulphur than phosphorus. These results indicate that sulphur would become a limiting factor before phosphorus.

It is generally concluded that sulphur to be available for plant food must be in the sulphate form, so that a soil having a high sulphur content may not necessarily supply enough sulphate sulphur for maximum growth. Brown and Kellogg (*6*) have shown that different soils have unlike sulphofying powers and some of the factors influencing the change of elemental sulphur and sulphides to sulphate form. In lysimeter experiments at Cornell, Lyon and Bizzell (*17*) report that the sulphate sulphur in the drainage water was from three to six times as great as in the crops and the sulphur content of the drainage water from the unplanted soil was about equal to the sulphur content of the crop and drainage water from the planted soil. Swanson and Miller (*26*) conclude from an investigation on sulphur in Kansas soils that—

> the loss in sulphur due to the amount taken up by the crop is insignificant as compared with the total amount which has disappeared from the soil. This means that sulphofication has been in excess of the needs of the crop, and the sulphates produced have been leached out of the ground.

[1] Reference is made by number (italic) to "Literature cited," pp. 100–102.

They report no increased yield when sulphur was applied as potassium sulphate (K_2SO_4), but state that the loss of sulphur can not continue without affecting crop yields. Hart and Peterson (*11*) calculate from data obtained at the Rothamsted Experiment Station, Harpenden, England, and the Wisconsin Experiment Station that the loss of sulphur in drainage water is three times that brought to an acre surface from the atmosphere.

Results of the investigations mentioned above show that the soil is capable of producing sulphate sulphur and that there is a tremendous loss of such sulphur in the drainage water. In certain cases no beneficial results have been obtained from sulphur fertilizers, which is not surprising, but in many instances sulphur application has caused increased yields. No doubt, in many soils, if the supply of sulphate sulphur formed was retained for plant food, they would not respond to sulphate treatment but the continual loss of sulphate sulphur and the large amount needed by some plants require that additional sulphate sulphur be present during the growing period to obtain better growth. A soil with a high sulphur content may not supply enough sulphate sulphur in comparison to the other plant food to obtain the maximum growth, while conditions in a soil of lower sulphur content may be such as to supply an adequate amount of sulphate sulphur.

In addition to sulphur acting as a plant food, several other explanations have been given as to its action in the soil. Certain investigators, Bernhard (*3*), Chancrin and Desriot (*8*), say that it functions as a partial sterilizer, others that the sulphuric acid produced acts upon the mineral matter of the soil, rendering it more soluble. Lipman (*15*, *16*) and his coworkers have shown (*1*) that the oxidation of sulphur in sand and soils has acted upon the raw-rock-phosphate so as to increase the water and ammonium-citrate-soluble phosphorus (*2*); that the formation of sulphate sulphur paralleled the increase of available phosphoric acid (*3*); and that the sulphur-floats-soil compost could be employed as a substitute for acid phosphate for plant growth. Brown and Gwinn (*5*) have found that the addition of sulphur to the soil increased the availability of raw-rockphosphate, the gain being greatest where manure and sulphur were used together. McLean (*18*) in a number of experiments has shown the conditions which are more favorable for the oxidation of sulphur by microorganisms and production of available phosphorus. Lipman (*14*) suggests that the sulphuric acid may act in making alkali areas productive by converting sodium carbonate into sodium sulphate. The favorable results obtained by adding gypsum have often been attributed to the calcium liberating potassium, but the experiments of Hart and Tottingham (*12*) show that a complete fertilizer plus calcium sulphate gave increased yields over those obtained with a complete fertilizer containing potassium chlorid, and that here the action of calcium sulphate must have been direct.

The action of sulphates upon soil bacteria has also been studied. Fred and Hart (*9*) have shown small increases in the number of soil bacteria and a slight increase in ammonification and carbon-dioxid evolution by adding certain sulphates to the soil. A bacteriological investigation by Janicaud (*13*) indicated that sulphur had a favorable influence on the development of bacteria in the soil. Pitz (*20*) reports no marked effect on the number of bacteria found on agar plates, but he does report an increase in legume bacteria from the use of calcium sulphate. Elemental sulphur caused a decrease in the total number of bacteria that grow on agar plates, but an increase in ammonification was accompanied by a parallel decrease in nitrate formation. Boullanger and Dugardin (*4*) state that the presence of small amounts of sulphur materially increases the activity of the ammonifying bacteria. Ames and Richmond (*2*) conclude from experimenting on relation of sulphofication to nitrogen transformation that the increase in ammonia which accompanied the decrease in yield of nitrates when sulphur was oxidized can not be considered as indicative of sulphofication having exerted a stimulating effect on ammonification. A deficiency of base in the soil allows the ammonia formed to neutralize the sulphur and it remains as ammonium sulphate.

Certain soils in Oregon have responded greatly to the use of sulphur and its compounds. In this locality greater crop production in many cases has resulted from using gypsum than in using lime. Reimer (*21*) of the Southern Oregon Experiment Station has obtained large increases in alfalfa yield by the use of elemental sulphur. This marked effect from the use of sulphur fertilizers suggested that it would be of interest and practical value to carry on some greenhouse experiments in order to study the effect of sulphur on early growth and composition of the plants, and also to attempt to determine whether it acted directly in supplying the plant with food or functioned in some other way.

PLAN AND OBJECT OF THE EXPERIMENT

For this work three Oregon soils, classified as a beaverdam, a Medford loam, and an antelope-clay-adobe, and designated as A, B, and C, respectively, in the tables, were chosen. The first was taken mainly for its high sulphur content, the second one because it did not respond to sulphur treatment in the field, while the third did respond to elemental sulphur fertilizer. The results obtained on analysis of these soils are given in Table I. The plants chosen were red clover, oats, and rape.

TABLE I.—*Percentage of constituents foun in soils*

Constituent.	Soil A.[b]	Soil B.[b]	Soil C.[b]
Potassium oxid (K_2O)	0.32	1.25	0.60
Phosphorus pentoxid (P_2O_5)	.349	.160	.135
Sulphur	.183	.034	.027
Calcium oxid (CaO)[a]		4.02	3.24
Calcium carbonate ($CaCO_3$)[a]		None.	.11

[a] Taken from analyses of soil sample when the field experiments were carried on.
[b] Soil A=beaverdam; soil B=Medford loam; soil C=antelope-clay-adobe.

It was decided to apply sulphur in the form of sodium sulphate (Na_2SO_4), calcium sulphate ($CaSO_4$), and elemental sulphur. The elemental sulphur was mixed with the soil at the time of planting, but the sulphates were added daily in the form of a solution. This daily addition of sulphates maintained a continuous supply of sulphates for the plant and it was thought that through the growth of the plants in the pots receiving the different forms of sulphur one could ascertain whether the elemental sulphur was able to supply the necessary sulphate. In order to eliminate nitrogen as a limiting factor, sodium nitrate ($NaNO_3$) in solution was added daily. By keeping up an available supply of nitrogen, a study could be made of the influence of sulphur fertilizer on the amount of nitrogen taken up by the plant. Nitrogen and sulphur enter into the composition of proteins and mustard oils so that an abundant supply of sulphates and nitrates in the plant may increase the elaboration of those organic compounds containing sulphur and nitrogen.

In addition to growing the plants on soil, they were also grown in sand pots receiving extract from the soil plus any nutrient that was added to the soil. For example, for a soil receiving a solution containing calcium sulphate and sodium nitrate, there was a corresponding sand pot receiving a water extract of the soil plus calcium sulphate and sodium nitrate. The following statement shows what each pot growing clover received:

SOIL

Pot 1:
- Calcium sulphate.
- Sodium nitrate.

Pot 2:
- Sodium sulphate.
- Sodium nitrate.

Pot 3:
- Sodium nitrate.
- Calcium carbonate.
- Sulphur.

Pot 4: Sodium nitrate.

Pot 5:
- Sodium nitrate.
- Calcium carbonate.

Pot 6: No fertilizer.

SAND

Pot 7:
- Soil solution.
- Calcium sulphate.
- Sodium nitrate.

Pot 8:
- Soil solution.
- Sodium sulphate.
- Sodium nitrate.

Pot 9:
- Soil solution.
- Sodium nitrate.
- Calcium carbonate.
- Sulphur.

Pot 10:
- Soil solution.
- Sodium nitrate.

Pot 4 (Pl. 9, A, B, and C) is a control to compare with 1 and 2, and 5 is a control on pot 3. Pot 10 receives no sulphur except that in the original soil extract. The pots growing oats and rape were treated in the same way, and this was repeated for each soil, making in all 90 pots (Pl. 9–12).

Hall, Brenchley, and Underwood (*10*) at the Rothamsted Experiment Station, in some experimental work in support of the theory of the direct nutrition of plants by fertilizers, found that wheat and barley showed parallel growth in the soil, in the soil extracts, and in artificial solutions of the same phosphorus-pentoxid (P_2O_5) and potassium-oxid (K_2O) content. The soil solutions corresponded to the natural drainage water, depending upon the past fertilizing treatment and present composition of the soil. The growth in extracts from poorly fertilized soils could be made equal by direct addition of suitable phosphate and potassium salts. Boiling did not affect the nutritive value of the solutions, and the diffusion of the nutrient solution over particles of sand did not interfere with the growth, although proper aeration of the roots was found to be essential.

By growing the plants on sand the possibility of the sulphates acting on minerals in the soil would be eliminated, and boiling the soil extract would destroy the bacteria originally in the solution. So in these experiments, if an increase in growth is observed in the soil pots from the application of sulphur fertilizer, and a corresponding increase is also obtained in the sand pots containing soil extract to which sulphur had been added, this same order of growth in the soil and sand would indicate in all probability that sulphur had acted directly in promoting the growth.

The object of this experiment was to make a study of the influence of an available supply of sulphates on the early growth of the plants and determine whether the elemental sulphur was capable also of supplying the necessary sulphates; to see what effect sulphates would have on the nitrogen content of the plant and if beneficial results are obtained whether the sulphur acted directly as a plant food in producing them.

EXPERIMENTAL WORK

The pots used were ordinary clay flowerpots which had been paraffined on the inside, and each contained about 700 gm. of soil. The sand was of a fine quality, obtained from Eimer and Amend. It was washed with dilute hydorchloric acid until no potassium, phorphorus, or sulphur was detected in the acid extract. Larger pots were not used on account of the beginning of this type of experiment, the number of pots needed and the individual attention required. The growing period was about two months with the exception of the oats which were allowed to ripen. The seed was sown on March 15, and the work was carried on in the greenhouse. At the end of a month the plants were thinned out so that there were 10 clover plants, 6 oat plants, and 3 rape plants per pot. At this time the sulphur and nitrogen were determined in the clover plants taken from soil A. The clover and rape were cut on June 1, the dry weight taken, and the total sulphur and nitrogen determined. Twenty-five cc. of nutrient solutions containing compounds as given in the statement on p. 90 were added daily to each pot after growth had started.

Where elemental sulphur and calcium carbonate ($CaCO_3$) were added, 0.3 gm. and 1 gm., respectively, of the above substances were mixed with the soil or sand in each pot before planting. In the control receiving no added nutrients, 25 cc. of distilled water was added. The concentration of the salts per liter of solution were as follows:

	Gm. per liter.
Sodium nitrate	0.25
Sodium sulphate	.20
Calcium sulphate	.25

The salts were dissolved in the same solution when more than one salt was added to a pot. The sand cultures received the soil extract which contained the additional nutrients as required. When the cultures required further moisture, the same amount of water was added to each of the pots. The soil extract was prepared by thoroughly mixing one part of soil with two of water. The solution was allowed to stand over night and then filtered through a porcelain filter. The clear filtrate was sterilized by boiling for 15 minutes. Analysis of the soil solution as given in Table II was made according to a method given by Stewart (*25*).

TABLE II.—*Soil constituents found in soil extracts expressed as parts per million of the soil extract*

Soil.	Potassium oxid.	Phosphorus pentoxid.	Sulphate sulphur.	Total sulphur.	Calcium oxid.
A [a]	10.0	3.2	7.4	9.6	23.0
B [a]	19.8	3.4	3.6	3.6	15.0
C [a]	7.2	1.0	3.2	3.2	22.0

[a] Soil A=beaverdam; soil B=Medford loam; soil C=antelope-clay-adobe.

DISCUSSION OF RESULTS

On examining the data in Table III it is noted that the weight of the straw grown on soils B and C and receiving sulphur fertilizer is greater than where no sulphur was used. An increase in weight of the crops is also observed in the sand pots receiving additional sulphur over those receiving soil extract plus sodium nitrate. The absence of plant food in the original sand and the use of sterilized soil extract shows undoubtedly that sulphur acted directly in promoting this growth. The same response of the soil to sulphur leads one to conclude that the sulphur here too has acted directly in promoting the growth. This increase in growth is also accompanied by an increase in sulphur content of the oat straw. In soil A this increase from sulphur application is not obtained. The weight of straw from the pots receiving calcium sulphate, sodium sulphate, and sulphur in addition to sodium nitrate is no greater than from the one receiving sodium nitrate only. In the sand pots receiving added sulphur we find no increase in weight of straw over the one receiving no extra sulphur. This is not surprising when the sulphur content

of the soil extracts is compared. The soil solution from A contains twice as much sulphate sulphur as the soil extracts from B and C and the total sulphur is three times as great. Apparently there is enough sulphur in the soil solution compared to the other elements for straw production. The development of the oat seed as shown in Table IV, agrees very well with the weight increases of the straw as shown in Table III. In comparing the growth of the oats on the soils receiving sodium nitrate plus calcium carbonate to those receiving sodium nitrate only, the calcium carbonate appears to have an injurious effect upon growth, but in comparing the calcium carbonate-sodium-nitrate-treated soils to those receiving sulphur, sodium nitrate, and calcium carbonate, the sulphur has caused increased growth in all cases.

TABLE III.—*Weight of oat straw and its percentage of sulphur and nitrogen on the different soils and sand cultures obtained from the different fertilizer treatment*

Treatment.	Soil.[a]								
	A.			B.			C.		
	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.
	Gm.			*Gm.*			*Gm.*		
Calcium sulphate..... Sodium nitrate.....	2.21	0.346	0.28	1.92	0.45	0.21	2.01	0.175	0.32
Sodium sulphate..... Sodium nitrate.....	2.13	.282	.33	1.90	.42	.266	2.01	.113	.33
Sulphur..... Sodium nitrate..... Calcium carbonate....	2.22	.370	.42	1.96	.58	.336	1.75	.30	.21
Sodium nitrate.....	2.23	.016	.17	1.41	.....	.476	1.68	.005	.54
Sodium nitrate..... Calcium carbonate....	1.83	.....	.29	1.34	.....	.672	.63	.....	.....
No fertilizer.....	.97	.143	.66	1.67	.....	.806	.54	.....	.49

Treatment.	Sand.[a]								
	Extract A.			Extract B.			Extract C.		
	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.
	Gm.			*Gm.*			*Gm.*		
Calcium sulphate..... Sodium nitrate.....	1.69	0.16	1.30	1.72	0.21	0.92	1.62	0.33	0.80
Sodium sulphate..... Sodium nitrate.....	1.63	.30	1.10	1.57	.13	.98	1.53	.34	.96
Sulphur..... Sodium nitrate..... Calcium carbonate....	1.48	.42	1.54	1.36	.32	1.29	1.76	.39	.73
Sodium nitrate.....	1.59	.13	1.50	1.06	.015	1.46	1.08	.05	1.70

[a] A=beaverdam soil or sand; B=Medford loam; C=antelope-clay-adobe.

TABLE IV.—*Weight of oat seed grown on soil and sand cultures*

[Percentage of nitrogen is given on oats grown in soils A and B]

Treatment.	Soil.[a]					Sand.[a]		
	A.		B.		C.	Weight.		
	Weight.	Percentage of nitrogen.	Weight.	Percentage of nitrogen.	Weight.	Extract A.	Extract B.	Extract C.
	Gm.		*Gm.*		*Gm.*	*Gm.*	*Gm.*	*Gm.*
Calcium sulphate Sodium nitrate	1.63	1.72	1.25	1.73	0.85	0.67	0.71	0.64
Sodium sulphate Sodium nitrate	1.44	1.79	1.13	1.71	.92	.77	.55	.48
Sulphur Sodium nitrate Calcium carbonate	1.45	1.46	.96	1.79	.82	.67	.38	.72
Sodium nitrate	1.62	1.73	.76	2.33	.61	.55	.36	.26
Sodium nitrate Calcium carbonate	1.06	1.77	.52	2.53	.08	.....	.....	.....
No fertilizer	.34	2.29	.27	2.33	.10	.....	.....	.....

[a] A=beaverdam soil or sand; B=Medford loam; C=antelope-clay-adobe.

The results on clover as given in Table V show increased yields in all cases where sulphur was added to the soils. This increase is also seen on the sand cultures receiving soil solutions from soils B and C, but not on the sand receiving nutrients as soil A. The oats grown on soil A, as on the sand receiving extract from A, did not respond to sulphur application, but the clover did respond to sulphur treatment of the soil. However, the clover growing on the sand and receiving its plant food from the soil extract did not show increased growth where sulphur was added. Naturally one may attribute this difference to some other factor present in the soil which was favorably influenced by the sulphur. Omitting this important factor and observing the weights of the dry material grown in the soil solution, there appears to be enough available sulphur in soil A and in the soil extract to eliminate sulphur as being directly a limiting factor as a plant food, while in the other soils the supply of available sulphur seems to be limited in comparison to the other plant food available.

It is probable that the other factors acting here are the legume bacteria which are present in the soil but not in the sand. The data in Table VI show the increase in weight of the roots where sulphur was used, and when the roots were examined the number of nodules, according to estimate, varied directly as the weight of the roots. The roots of those plants grown in the sand, of course, contained no nodules. Another reason why the bacteria appear to be favorably influenced by the sulphur is the noticeable percentage of increase of nitrogen in those plants grown on soil receiving sulphur while the plants grown on sand do not show this increase in nitrogen content. For this short period of growth the sulphur apparently has a marked influence on the nitrogen content of the clover.

TABLE V.—*Weight of clover and its percentage of sulphur and nitrogen on the different soils and sand cultures obtained from the different fertilizer treatments*

Treatment.	Soil.[a]								
	A.			B.			C.		
	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.
	Gm.			*Gm.*			*Gm.*		
Calcium sulphate	3.98	0.197	2.94	2.20	0.227	2.78	1.69	0.240	2.67
Sodium nitrate									
Sodium sulphate	4.06	.181	2.93	1.37	.280	2.95	1.66	.205	3.33
Sodium nitrate									
Sulphur									
Calcium carbonate	3.89	.198	3.10	1.87	.234	3.64	.65	.....	1.28
Sodium nitrate									
Sodium nitrate	1.76	.097	2.32	.99	.056	2.19	.62	.....	2.10
Calcium carbonate	1.48	.012	1.98	.61	.032	1.92	.42	.....	1.79
Sodium nitrate									
No fertilizer	1.80	.055	1.91	.65	.037	2.58	.49	.....	1.62

Treatment.	Sand.[a]						
	Extract A.		Extract B.		Extract C.		
	Weight.	Percentage of nitrogen.	Weight.	Percentage of nitrogen.	Weight.[b]	Percentage of sulphur.	Percentage of nitrogen.
	Gm.		*Gm.*		*Gm.*		
Calcium sulphate	0.54	2.38	0.38	1.8	1.0	0.467	2.11
Sodium nitrate							
Sodium sulphate	.58	2.56	.36	2.14	.....	.....	.....
Sodium nitrate							
Sulphur							
Calcium carbonate	.49	2.54	.24	1.92	1.20	.493	2.42
Sodium nitrate							
Sodium nitrate	.63	2.35	.22	1.90	.52	.....	1.86
Calcium carbonate							
Sodium nitrate	.....	.....	.....	.....	.....	.....	.....
No fertilizer	.....	.....	.....	.....	.....	.....	.....

[a] A=beaverdam soil or sand; B=Medford loam; C=antelope-clay-adobe.
[b] Plants were not thinned out. There was no growth in sodium-sulphate sodium-nitrate pot.

It appears that the sulphur increases the nitrogen content by stimulating the activity of the legume bacteria causing greater nitrogen fixation. The total nitrogen removed from the sulphured soils is three times as great as from the unsulphured soils. These plants, of course, have grown for only two months and whether the mature plant would show this same ratio will have to be decided by further experiments. In comparing the nitrogen and sulphur contents of the clover grown in soil A at two different periods as given in Table VII, there is a decrease in percentage of nitrogen and sulphur from May 1 to June 1. Perhaps the percentage

of total sulphur and nitrogen would grow less as the plant developed, until, at maturity, the nitrogen content would average about the same for all the clover grown under the different fertilizer treatments. In certain pots, maturity, undoubtedly, would be reached sooner, but allowing each group to grow until they all reached the same stage of development, it would be of importance to know whether the sulphur had affected the nitrogen content and the character of the compounds containing nitrogen. Samples of alfalfa grown on sulphur-fertilized soils in Oregon,[1] as shown in Table IX, have shown a higher nitrogen content than those grown on the same soil without sulphur application. Shedd (*24*) reports increase in protein content of soybeans from ammonium-sulphate fertilizer and Ames and Boltz (*1*) report larger protein content in rape where sulphates were in the fertilizer used. In this experiment available nitrogen was present in the form of nitrates. It would be of interest to know whether by maintaining the sulphate supply an increase in nitrogen assimilation from the air could be brought about.

TABLE VI.—*Weight of clover roots expressed in gms. grown on the different soils receiving various treatments*

Treatment.	Soil A.[a]	Soil B.[a]	Soil C.[a]
Calcium sulphate Sodium nitrate	1.15	0.85	0.44
Sodium sulphate Sodium nitrate	.95	.36	.28
Sulphur Calcium carbonate Sodium nitrate	.93	.49	.10
Sodium nitrate	.60	.33	.18
Sodium nitrate Calcium carbonate	.53	.23	.10
No fertilizer	.51	.21	.13

[a] Soil A=beaverdam; soil B=Medford loam; soil C=antelope-clay-adobe.

TABLE VII.—*Sulphur and nitrogen content at different stages of growth in clover grown on soil A*

Treatment.	Percentage of sulphur, May 1.	Percentage of sulphur, June 1.	Percentage of nitrogen, May 1.	Percentage of nitrogen, June 1.
Calcium sulphate Sodium nitrate	0.285	0.197	3.14	2.94
Sodium sulphate Sodium nitrate	.260	.181	3.57	2.93
Sulphur Sodium nitrate Calcium carbonate	.360	.198	3.24	3.10
Sodium nitrate	.086	.097	2.80	2.32
Sodium nitrate Calcium carbonate	.070	.012	2.13	1.98
No fertilizer	.130	.055	2.67	1.91

[1] Reimer and Tartar. Unpublished data, Oregon Agricultural Experiment Station.

The rape plant did not show this general response to sulphur treatment like the other plants, for in several instances the growth is greater in those pots receiving no sulphur. However, if a comparison is made between the soil and sand pots receiving sodium nitrate and those receiving sodium nitrate plus sodium sulphate, it is observed from the data in Table VIII that increased growth has resulted from the addition of sodium sulphate on both the sand and the soil. The growths of rape on the soils and their extracts parallel each other very well. The rape grew very poorly on the extract from soil C, so no data are given. Where a comparison is made on the growths of the crops on the different soils they do not follow the same order, and the sulphur and nitrogen content do not show the same change from the different fertilizer treatments; but the rape grown in pots receiving sulphur, sodium nitrate, and calcium carbonate has a higher percentage of sulphur than that grown in the other pots, yet the total sulphur removed is not much larger. The plants grown on the sand have higher percentages of nitrogen and sulphur but the total sulphur and nitrogen removed is no greater than for those plants grown in the soil.

The total sulphur present in plants is far greater where sulphur fertilizer was used. On account of the small amount of material, the sulphate sulphur was not determined so that it is not possible to tell whether the organic sulphur was increased. Analysis, in this laboratory, for organic sulphur and sulphate sulphur in alfalfa hay grown on soils receiving 300 pounds of sulphur per acre and on the same soils receiving no sulphur fertilizer as given in Table IX shows that the organic sulphur was increased by the application of sulphur. Shedd (*24*) found an increase in organic sulphur in soybeans from the use of ammonium sulphate fertilizer.

TABLE VIII.—*Weight of rape and its percentage of sulphur and nitrogen on the different soils and sand cultures*

Treatment.	Soil.[a]								
	A.			B.			C.		
	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.
	Gm.			*Gm.*			*Gm.*		
Calcium sulphate.....	1.81	0.75	1.39	1.57	0.66	0.903	1.98	0.61	1.06
Sodium nitrate.....									
Sodium sulphate.....	2.92	.50	1.65	2.26	.55	.990	2.31	.65	.99
Sodium nitrate.....									
Sulphur.....	1.03	1.19	1.29	1.89	.80	1.47	1.73	.81	1.74
Sodium nitrate.....									
Calcium carbonate....									
Sodium nitrate.....	1.46	.057	1.57	1.79	.024	1.43	1.98	.02	1.36
Sodium nitrate.....	1.89	.051	.84	1.61	.017	1.45	.59	.....	3.00
Calcium carbonate....									
No fertilizer.....	.71	.236	1.18	.75	.022	1.24	.30	.....	1.20

[a] A=beaverdam soil and sand; B=Medford loam; C=antelope-clay-adobe.

TABLE VIII.—*Weight of rape and its percentage of sulphur and nitrogen on the different soils and sand cultures*—Continued.

Treatment.	Sand.[a]					
	Extract A.			Extract B.		
	Weight.	Percentage of sulphur.	Percentage of nitrogen.	Weight.	Percentage of sulphur.	Percentage of nitrogen.
	Gm.			*Gm.*		
Calcium sulphate Sodium nitrate	0.88	1.13	3.53	0.73	0.90	3.75
Sodium sulphate Sodium nitrate	1.03	.93	2.35	.62	1.40	3.38
Sulphur Sodium nitrate Calcium carbonate	.50	1.29	8.29	.35	1.51	5.58
Sodium nitrate	.59	.07	4.31	.25	.....	4.00
Sodium nitrate Calcium carbonate	.....	.....	.....	.....	.....	.....
No fertilizer	.....	.....	.....	.....	.....	.....

[a] A=beaverdam soil and sand; B=Medford loam; C=antelope-clay-adobe.

Petersen (*19*) in an analysis showing different forms of sulphur in plants found more organic sulphur in clover, rape, and radish where sulphur was present in the fertilizers used, and Ames and Boltz (*1*) report increase of organic sulphur in rape where sulphates were applied to the soil. These results and the increase in nitrogen content support the idea that maintaining a sufficient supply of sulphate sulphur and available nitrogen in the soil would tend toward more protein or other sulphur organic-compounds being formed in the plant. The sulphur content is generally increased wherever sulphur fertilizer is added. The sulphate radical is in combination with some other radical and the question arises whether the mineral content or ash of the plant is not increased by this noticeable increase of sulphate sulphur. If sulphur is applied as sodium sulphate will the sodium content of the plant be increased or if calcium sulphate is used will the calcium be absorbed by the plant?

TABLE IX.—*Percentage of total sulphur, sulphate sulphur, organic sulphur, and total nitrogen in alfalfa grown on sulphured and unsulphured portions of three different Oregon soils*

Soil.	Treatment.	Total sulphur.	Sulphate sulphur.	Organic sulphur.	Total nitrogen.
1	Sulphur	0.227	0.0603	0.167	2.51
	No sulphur	.127	None.	.127	2.22
2	Sulphur	.167	.0356	.131	2.16
	No sulphur	.118	None.	.118	2.01
3	Sulphur	.200	.059	.141	2.38
	No sulphur	.118	None.	.118	2.09

Soil B responds to sulphur treatment in these pot tests while in the field elemental sulphur caused no increase in production. In the analysis of the soils in Table I soil C contains calcium carbonate, while B does not. It may be that the sulphur was oxidized in the field as in these pot tests, but as no base was present to combine with the sulphuric acid, the latter interfered with the growth. In C, calcium carbonate was present which neutralized the acidity and provided sulphates which produced the beneficial effects. Data in Table X show the difference in sulphate content between the soils receiving calcium carbonate and sodium nitrate compared to those receiving the above named compounds plus sulphur. The results show that the elemental sulphur was oxidized to the sulphate form. Furthermore, no weighable quantities of barium sulphate were obtained from the water extracts of the unsulphured soils, showing a deficiency of sulphate sulphur for immediate plant use. The rate of sulphofication appears to be greater in the beaverdam soil than in the other soils containing less organic material. While soil A has a high sulphur content and also readily oxidizes elemental sulphur, it gave a noticeable response to sulphate teatment when clover was grown.

TABLE X.—*Sulphur as sulphate in the water extract from 40 gm. of soil A and 80 gm. each of B and C after growth of plants*

[Weight in milligrams]

Treatment.	Soil A.[a]			Soil B.[a]		Soil C.[a]	
	Clover.	Oats.	Rape.	Clover.	Oats.	Clover.	Oats.
Sulphur..... Calcium carbonate... Sodium nitrate.....	21.9	34.4	25.9	4.1	10.8	10.3	11.2
Calcium carbonate.... Sodium nitrate.....	None.	None.	None.	None.	None.	None.	None.

[a] Soil A=beaverdam; soil B=Medford loam; soil C=antelope-clay-adobe.

It is realized that the experiments conducted here have not been on a large scale and the conditions are not comparable to those in the field. No general conclusions can be made, but what conclusions are drawn apply only to the limits of this experiment and based upon conditions of this work where each individual case can be compared to the other. This work will be repeated on a larger scale and expanded so as to answer some of the questions which have arisen during this experiment.

SUMMARY

1. Pot experiments to show the effect of sulphur fertilizers—namely, sodium sulphate, calcium sulphate and sulphur on red clover, rape, and oats were carried with three different soils, including one with a high sulphur content, one that did not respond to elemental sulphur in the field, and one that did.

2. To eliminate the sulphur compounds acting upon the insoluble plant food and soil organisms, these plants were also grown on sand receiving the sterilized soil extract and certain pots received the additional sulphur fertilizers as the soil.

3. Sodium sulphate and calcium sulphate were added daily in solution form. The elemental sulphur was mixed with the soil and calcium carbonate at the time of sowing the seed.

4. Sodium nitrate solution was added daily to eliminate available nitrogen as a limiting factor of growth and also to determine what effect sulphates would have on nitrogen assimilation by the plant.

5. The plants were grown for two and one-half months and the dry weights of the tops were recorded. The total sulphur and nitrogen was determined in the majority of cases.

CONCLUSIONS

1. Addition of sulphate and elemental sulphur enhanced the growth of the plants grown in pots in the greenhouse.

2. The corresponding increases obtained on the soil extracts indicated that sulphur acted directly in promoting this growth.

3. The great increase in the nitrogen content of the clover grown on the soil where sulphates had been added is the result in all probability of the sulphates stimulating the action of the legume bacteria.

4. Sulphates caused increased root development and number of nodules on the clover grown in the soil pots.

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PLATE 9

A.—Clover on soil A. The top row, reading from left to right, shows the soil pots which received the following fertilizers:

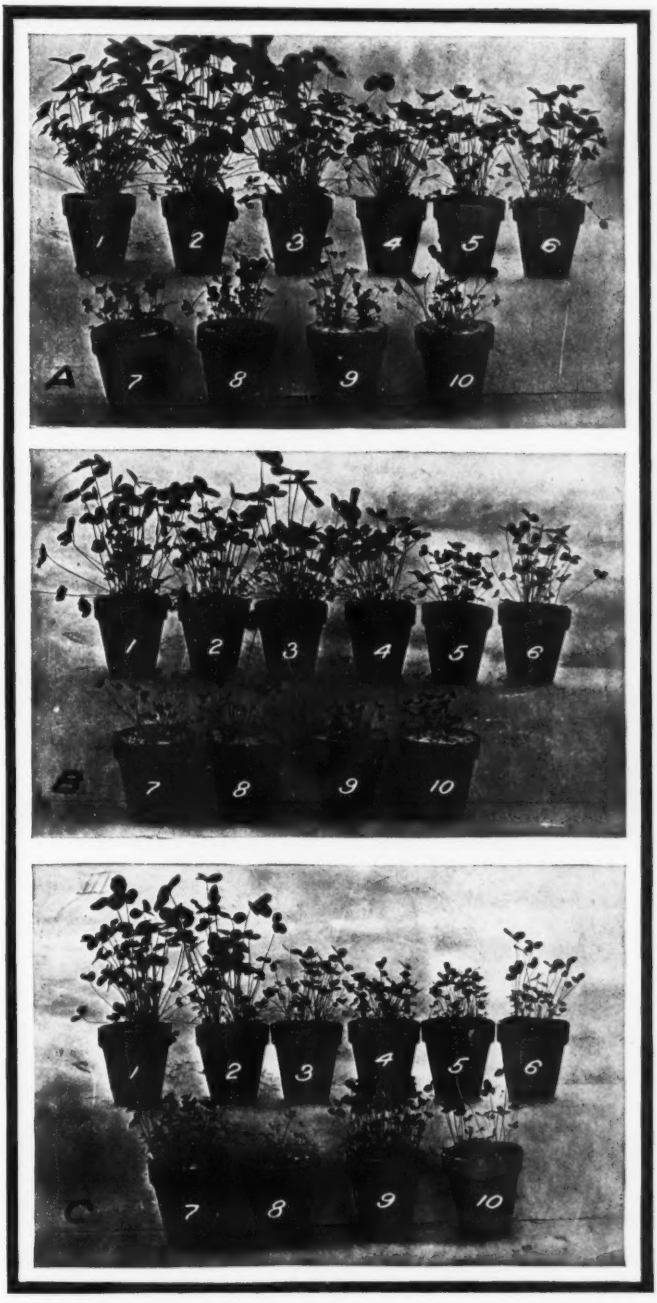
Pot 1, calcium sulphate, sodium nitrate; pot 2, sodium sulphate, sodium nitrate; pot 3, sulphur, sodium nitrate, calcium carbonate; pot 4, sodium nitrate; pot 5, sodium nitrate, calcium carbonate; pot 6, no fertilizer.

The lower row, reading from left to right, shows the sand pots which received the following fertilizers:

Pot 7, calcium sulphate, sodium nitrate; pot 8, sodium sulphate, sodium nitrate; pot 9, sulphur, calcium carbonate, sodium nitrate; pot 10, sodium nitrate.

B.—Clover on soil B. The top row, reading from left to right, shows the soil pots which received the same fertilizers as in series A above. The lower row, reading from left to right, shows the sand pots which received the same fertilizers as in series A.

C.—Clover on soil C. The top row, reading from left to right, shows the soil pots which received the same fertilizers as in series A. The lower row, reading from left to right, shows the sand pots which received the same fertilizers as in series A.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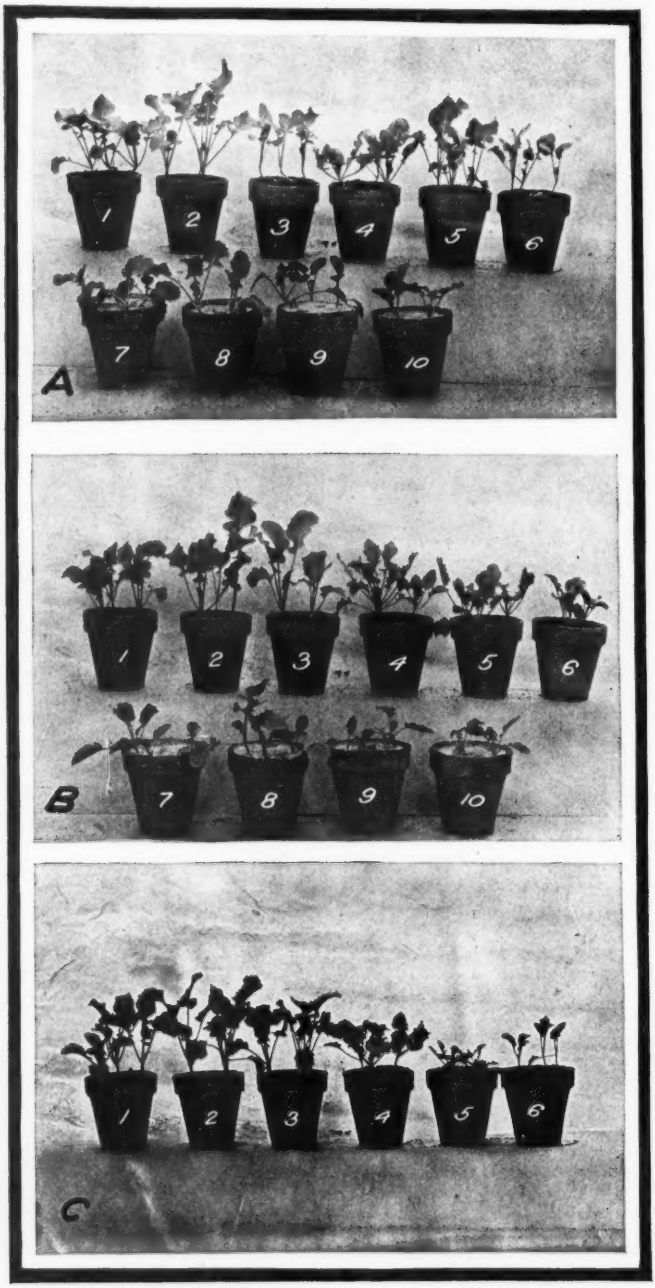
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PLATE 10

A.—Rape on soil A. The top row, reading from left to right, shows the soil pots which received the same fertilizers as in Plate 9, series A. The lower row, reading from left to right, shows the sand pots which received the same fertilizers as in pots in Plate 9, series A.

B.—Rape on soil B. The top row, reading from left to right, shows the sand pots which received the same fertilizers as in pots in Plate 9, series A.

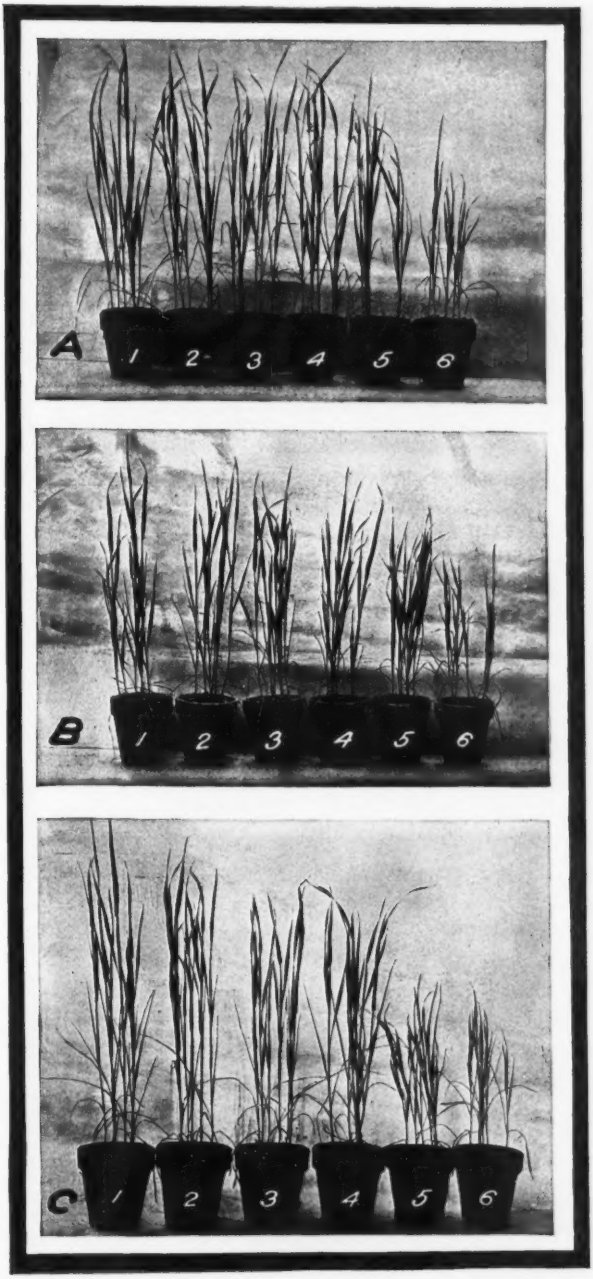
C.—Rape on soil C. The soil pots, reading from left to right, received the same fertilizers as in pots in Plate 9, series A.

PLATE 11

A.—Oats on soil A. The soil pots received the same fertilizers as in pots shown in Plate 9, series A.

B.—Oats on soil B. The soil pots received the same fertilizers as in pots shown in Plate 9, series A.

C.—Oats on soil C. The soil pots received the same fertilizers as in pots shown in Plate 9, series A.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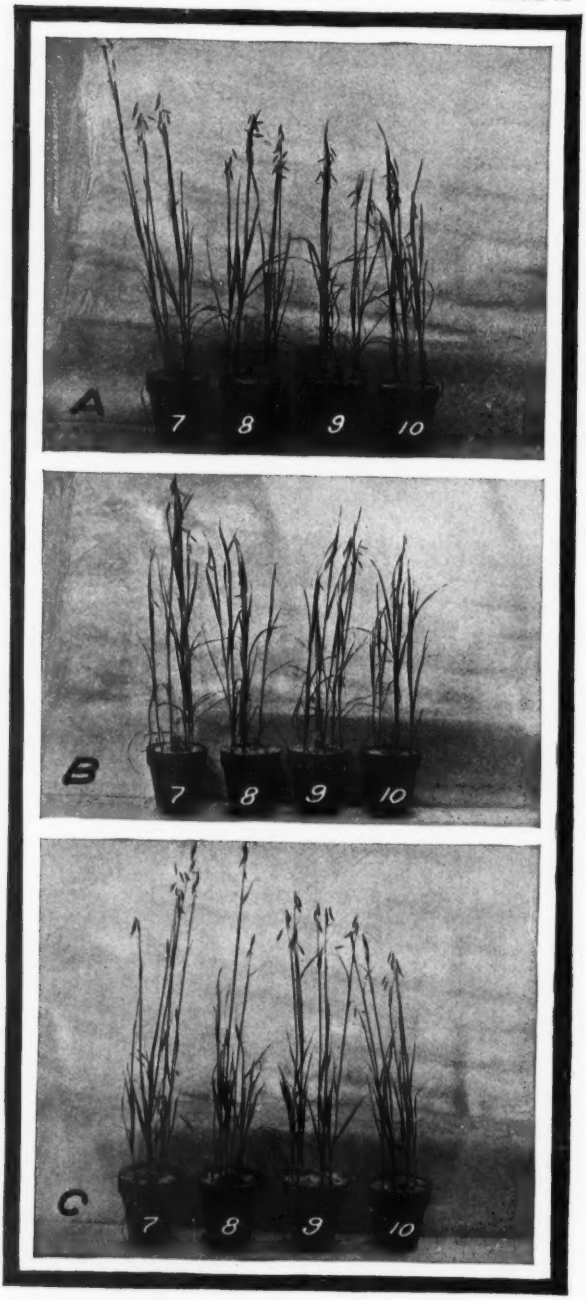
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PLATE 12

A.—Oats on sand cultures from soil A. The sand pots received the same fertilizers as in pots shown in Plate 9, series A.

B.—Oats on sand cultures from soil B. The sand pots received the same fertilizers as in pots shown in Plate 9, series A.

C.—Oats on sand cultures from soil C. The sand pots received the same fertilizers as in pots shown in Plate 9, series A.

RELATION OF WEATHER TO FRUITFULNESS IN THE PLUM[1]

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Under suitable growing conditions the plum tree is remarkable for the uniformity with which it annually produces a crop of flower buds. Bearing a full crop of flower buds annually, however, does not insure a full crop of fruit annually; therefore, it is evident that a considerable number of flowers fail to set fruit.[2] From the standpoint of fruit production, thinning, up to three-fourths of the bloom, is actually beneficial, but beyond this the margin is approached where the thinning process reduces the yield and there is economic loss. The status of setting in controlled crosses known to be fertile under tents was similar to that in the orchard generally. This general condition led to an attempt to isolate those factors of the weather influencing the setting of fruit which result in such great differences as a complete crop failure one year and an overproduction of fruit another.

The elements of what is commonly known as "weather" which have a bearing upon pollination and fertilization are wind, temperature, sunshine, and rain. The combinations of these most favorable to the setting of fruit are sunshine, a relatively high temperature, slight or no wind, and an absence of rain. It is apparent that certain weather conditions, good and bad, go together, but temperature and rain are undoubtedly the most important elements considered from the standpoint of the setting of fruit and will be given greatest emphasis.

The following statements may be regarded as fairly typical of the conception of the influence of unfavorable conditions at bloom. Cold weather, rain, poor locality, and severe cold winter weather are mentioned by Goff (*4*) [3] as inhibiting fruitfulness. Bad weather at flowering time has an "injurious influence on fruitage" by keeping away insect visitors and affecting the fecundation of the flowers (*15*). Damage to flowers by wind, hail, rain, insects, and fungi are commonly mentioned. Lord (*11*) states that all varieties when in bloom are extremely sensitive to cold or wet weather. Heideman (*9*) notes that ample cross-fertiliza-

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[2] "Setting of fruit" is a term in common use among fruit growers. In general, it is used in referring to the number of pistils which are swelling or "setting" six weeks or so after bloom. A distinction is made in common usage between the percentage of fruit to set and the percentage of a crop, in that the latter is used in speaking of mature fruit.

[3] Reference is made by number (*italic*) to "Literature cited," p. 125–126.

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tion does not prevent great differences in the crop from year to year. Some growers hold that there is a good fruit crop only during seasons with favorable weather for bees at blooming time. Hedrick (7) analyzed the weather records of New York with respect to fruit production and showed that in general unfavorable weather is the dominant factor in crop failures. In fact, for a long time fruit growers have recognized certain weather combinations as detrimental to or prohibiting the setting of fruit.

If weather is to be assigned such an important rôle in relation to fruit fulness, the question arises as to the significance of the great variation in the time of bloom from year to year. For instance, plums have varied nearly one month in the time of flowering at the Fruit-Breeding Farm in the last seven years, the earliest bloom in this period beginning April 24, 1915, and the latest May 20, 1916. The cause for such a variation in time of bloom should not be assigned entirely to the weather of early spring, because Sandsten (*13*) found, upon analyzing the blooming records at Madison, Wis., that the time of flowering was influenced more by the growing conditions of the preceding summer and fall than by those of the spring. In Plate 15 the prevailing weather of early spring when plums are in flower is presented in some detail. It will be seen from the analysis presented in these graphs that cool weather and frequent rains can be expected in Minnesota for a period of even greater length than that covered by the greatest extremes in the time of bloom. Therefore, inasmuch as a range in blooming time of as much as one month has not meant an escape from periods of unfavorable weather, early or late blooming does not necessarily have a constant relation to fruitfulness.

The period of 10 days after bloom was selected (Pl. 15) because it covers for the most part the time of fertilization. In only 10 instances out of 142 did the minimum temperature occur in the day and the maximum at night, so that the curve of maximum temperature may be considered as the day temperature and that of the minimum as the night temperature. In the graph for each season the period of bloom is indicated by the lighter shaded portion between the maximum and minimum temperature curves. In the case of wind and the character of the day (sunshine or cloudiness) a 12-hour day was taken because of the bearing of wind and sunshine on bee flight. The date in the graph is located in the midpoint, which is 12 m. The short, broken-line curves indicate the wind velocity during the daytime only, i. e., from 6 a. m. to 6 p. m. The legend is at the right of the graph. The character of the day is shown by the shading at the base of each graph; the dark bar represents the portion of the day which was cloudy, the cross bar that which was partly cloudy, and the white the time of sunshine. A dotted line is drawn through each graph at the 40° and 51° F. points, the former

being the point Goff (*5*) found that plum pollen did not germinate and the latter the temperature of slow tube growth.

Since the weather at the Fruit-Breeding Farm has not been recorded, this analysis is made from the records furnished by Mr. U. G. Purssell, of the United States Weather Bureau, at Minneapolis.

EFFECT OF UNFAVORABLE WEATHER ON THE SETTING OF FRUIT

It has been a matter of common observation among fruit growers that when the blooming period is accompanied by a prolonged rain there is generally a light setting of fruit. Halsted (*6*), in an attempt to determine the cause of this, performed an experiment in which an apple tree was kept wet with a spray of water for six days while in bloom. The weather was fair during the experiment. The sprayed tree failed to set any fruit, except in a few instances on the upper branches, while the surrounding trees of the same variety set full.

Beach and Fairchild (*3*) performed a similar experiment with a Mount Vernon pear tree and a Duchess grapevine. The pear tree subjected to a spray for nine days bore a single fruit. Pollen taken from "fresh anthers" on the fifth day and placed in a sugar solution proved to be "perfectly capable" of germination. Many of the stigmas examined 24 hours after the experiment began were found to be "dusted with pollen," although no insects had been seen near the tree. After the close of the experiment many anthers opened and shed an abundance of pollen.

In the case of the Duchess grape, although the 12 days' treatment did not cover the entire period of bloom, the treated vines bore many aborted berries, but on none of the clusters were all of the berries aborted. Also, the average size of the fruit was reduced approximately one-half.

In these experiments the conditions which generally accompany a prolonged rain were not duplicated exactly, and consequently other factors may have entered into the results obtained. However, a constant spray was effective in preventing fruitfulness in the apple and pear, and even in the case of the grape sufficient pollination to account for the setting of fruit which took place may have occurred after the water was turned off.

It will be of interest here, after a review of the experiments of Halsted (*6*) and of Beach and Fairchild (*3*), to include a statement concerning the percentage of fruit to set in a plot of Surprise seedlings at the University Farm in order to show the general effect of unfavorable weather. All trees bloomed heavily during the seasons of 1917 and 1918 and for this reason present an excellent illustration of the effect of weather upon the setting of fruit. These seedlings are about 13 years old, fairly uniform in size, and are growing under clean cultivation. It would appear that ample pollination would take place if the weather were favorable, because these seedlings are located within less than a quarter of a mile of the University apiary of about 100 colonies. In general it may be

stated that during both seasons conditions were unfavorable for insect flight. The weather conditions at time of blooming for these two seasons are shown in Plate 15.

TABLE I.—*Comparison of fruit setting in an orchard of 226 Surprise seedlings during the two relatively unfavorable seasons of 1917 and 1918* [1]

Range in percentage of fruit to set in 1917.	Range in percentage of fruit to set in 1918.						Total number of trees.
	0	1	5	10	20	30	
	Number of trees.	*Number of trees.*	*Number of trees.*	*Number of trees.*	*Number of trees.*	*Number of trees.*	
0	3	1	.....	.....	.....	.....	4
1	5	8	.....	4	3	.....	20
5	6	9	11	14	6	4	50
10	4	13	6	9	.....	3	35
20	17	22	12	13	14	.....	78
30	12	9	2	3	3	3	32
40	3	3	.....	.....	.....	1	7
Total number of trees	50	65	31	43	26	11	226

[1] The percentage set is based upon the total number of flowers borne. Each tree is placed in the table with reference to the percentage of fruit set in 1917 compared with that in 1918. For instance, in 1917 there were 78 trees in which 20 per cent of the flowers set, but in 1918 the set on these same trees ranged from 0 to 20 per cent.

The data are presented in the form of a correlation table in order to show the influence of heavy fruiting during one year upon the crop the succeeding year. Accordingly, each tree is placed in the table with reference to the percentage of fruit set in 1917 compared with that set in 1918.

Three things are outstanding in Table I: (1) The heavy setting or bearing of 1917 was shown to have no distinct influence on the succeeding crop in 1918; (2) there was a heavier setting in 1917 than in 1918, the relative number of trees setting below 20 per cent being 109 and 189, respectively; and (3) since by actual count it was determined in the 6-weeks period after blooming that only one pistil in four set or persisted on those trees bearing what was arbitrarily regarded as a "full set," it will be seen that many of the trees set an unusually small number of fruits, too few, in fact, to produce a full crop after allowing for subsequent loss. This condition is not unusual in the plum when blooming time is accompanied by unfavorable weather. The light set in those trees which produced normal flowers in abundance presents a condition quite similar to that which prevailed both seasons in a number of standard varieties and other seedlings under cultivation. In Plate 13, A and B, the contrast between the number of flowers borne and the fruit to set is shown.

ANALYSIS OF WEATHER AT BLOOMING TIME

With weather apparently having such an important bearing upon the setting of fruit, as is indicated in the spraying experiment and in Table I, a more detailed analysis of weather has been made during blooming time and for 10 days after, with the object of determining whether there are certain conditions each season which can be singled out as prohibiting a set of fruit. At the outset it should be stated that there are factors which operate beyond the 20-day period to reduce the crop. Nevertheless, there are influences entering during blooming time which do not operate in the same manner anywhere else in the life cycle. As a result of the sum total of these influences a sufficient number of pistils have or have not set, as the case may be, at the 5- or 6-week period to determine definitely the prospect of a crop.

WIND

The experiments of Waugh (*16*) show that no fruit set from wind-carried pollen when insects were excluded by a covering of coarse mosquito netting. Further tests (*18*) with microscopic slides covered with vaseline, to which pollen adheres, showed that when the slides were placed at various heights and distances from trees in full bloom on bright sunny days even a direct wind did not carry sufficient pollen to bring about effective pollination at a distance equal to that from one tree to another. Wind pollination, therefore, may be regarded as insufficient, even under the most favorable conditions.

Pollination under orchard conditions is affected by windy weather, however, especially when prolonged, if insect visits are prevented. During a strong wind, rain, cold, or cloudy weather, conditions are such that insect activity is reduced to a minimum. Waugh (*16*, *17*) shows that honey bees, of the 30 or more species of insects found to visit the plum, are (*16*, p. 247) "nearly always the most active workers, and the ones which, by the character of their operations in the flower, may be held chiefly responsible for the proper distribution of pollen." These results are confirmed by Backhouse (*1*). Wind, therefore, may be regarded as having more of an indirect than direct bearing upon the setting of fruit, since pollen is not wind-carried in quantities sufficient for ample pollination. The influence upon bee flight, however, may be serious at certain times.

The curve for wind in Plate 15 runs through the point of hourly wind movement from 6 a. m. to 6 p. m. While the average wind movement considered aside from sunshine and the character of the day is of little significance, it shows what may be expected at this time of year in Minnesota. The average wind movement per hour, within the above limits, for 7 years was approximately 15 miles, while the average of the extreme wind movement recorded, within the same limits, for the 7-year period was near 19. The extreme movement recorded was 38 miles. Assuming that a wind of 25 miles per hour approaches a condition where bee

flight is hindered, it will be seen from Plate 15 that wind alone is not generally prohibitive of bee blight, but that at certain critical times, as on April 28 and 29, 1915, following a period of cloudy weather with frequent rains, it may become important—more so, in fact, from the standpoint of insect flight than from that of mechanical injury to flowers.

In addition to the considerations noted above, wind has a general drying effect upon the flower parts. Dehiscence is quickened and petals drop earlier. There is, however, no marked drying noticeable in the stigma during early receptiveness, but late in the receptive period stigmas can be found which appear distinctly dry even before the stigmatic cells are dead. Since the absorption of stigmatic fluid is no doubt the first act in germination the drying effect of wind upon stigmas may be regarded as much more critical late in receptiveness than earlier, especially in view of the more unfavorable conditions for tube growth, if pollination has been delayed.

TEMPERATURE

Temperature is primarily of interest in this connection from three standpoints: (1) Its direct effect upon pollen or pistil, (2) its influence upon pollen-tube growth, and (3) its interference with bee flight. From Plate 15 it will be seen that there are many periods of low temperature during blooming time which are occasionally accompanied by frost. With reference to direct injury, it will be interesting to record here the damage to flowers at two distinct stages of growth.

On the night of April 19, 1918, a freeze occurred at the Fruit-Breeding Farm, when the petals were just showing in the earliest blooming varieties. There was no injury to pollen or pistil, but as many as one-half of the petals were killed on some of the varieties. These bloomed, however, at the usual time, and the small dead petals persisted, while those not killed underwent the usual enlargement.

This freeze was followed by another on May 12, one week after blooming, when the flowers were further advanced. But this time all stigmas were dead on the varieties which had bloomed earlier. The calyx tube was still persistent, as there had not as yet been sufficient pistil growth to break it except in two varieties of *Prunus nigra*. Although generally there was little injury to pistils at this stage, different varieties showed considerable differences in the degree of injury. On Stella, growing in a low location, approximately 65 per cent of the pistils were killed, and on Minnesota No. 21 (Burbank × Wolf), adjacent, there was less than 1 per cent. Where injury occurred the entire pistil was killed, and in two days it turned black, dried rapidly, and dropped a few days later at the pedicel base. On the higher locations there was no injury to any of the varieties. Compared with the region in Utah in which Ballantyne (2) studied frost injury, frosts do not appear to bear such a vital relation to fruitfulness in Minnesota.

Pollen taken from flowers in which the pistils were killed appeared normal in color and in content when observed in a mount of lactic acid. Its viability, however, was not tested, but judging from appearances this freeze injured pollen much less than pistils.

Goff (*5*) shows that plum pollen was not destroyed by a short exposure to freezing temperatures. Sandsten (*14*) tested this point further and found that when plum pollen was exposed to a temperature of 29.3° F., 56 per cent germinated, compared with 62 per cent in the check, which was not exposed to the freezing temperature. He also found that the time required for germination was increased one-half as a result of the influence of the low temperature. On the other hand, 21 plum pistils exposed to the same temperature for six hours were all killed except two.

The action of low temperatures in retarding pollen-tube growth is no doubt one of the primary factors in the failure of fruit to set. The experiments of Goff (*5*) show that plum pollen does not germinate at temperatures below 40° F., and even at temperatures as high as 51° F. that there is slow pollen-tube growth. A dotted line is drawn through the graph for each year in Plate 15 at these two points. The extent to which the curve for the minimum temperature extends below the line where pollen-tube growth does not take place shows that in some seasons, as in 1915, a prolonged cool period following blooming may be the principal cause of the failure of fruit to set.

With reference to the influence of temperature upon insect flight, it appears that a definite point can not be selected below which activity ceases. Furthermore, temperature can not be considered separate from wind, rain, and sunshine. Recent investigations upon the honey bee, which is the chief pollinizer of the plum, however, show something of its response to temperature. Phillips (*12*) states that 57° F. is "the lowest temperature which normal bees ever experience in the hive." At air temperatures below this immediately surrounding the bees in cold weather, they begin to cluster. Kenoyer (*10*) in reporting the data collected over a 29-year period by J. L. Strong at Clarinda, Iowa, shows that only 1 per cent of the total honey produced for that period was collected when the temperature was below 70° F. compared with 53 per cent when the temperature ranged between 80° and 90° F. Nevertheless, this does not deal directly with the point as to what temperature prevents the pollinating activity of bees on plums in early spring.

The opinions of two bee men regarding the lower temperature in which bees will fly are as follows:

> The normal temperature for bees to take flight is 46° F. This temperature is 1 degree to 2 degrees lower for Carniolan races and up to 3 degrees lower after long confinement.[1] The individual bee can continue muscular movement only so long as the temperature of the body does not fall below 45° F., but at this temperature it loses its power of movement. (*12*, p. 59.) In general bees will not

[1] Letter from Prof. Frances Jaeger, University Farm, Dec. 31, 1918.

fly from the hive until the temperature is about 60° F. unless they are inpelled to fly by a long period of confinement resulting in an accumulation of feces.[1]

The minimum temperature curves in Plate 15 show that there are only relatively short intervals when the temperature is below 50° F. It would appear that if bees were present in sufficient numbers, other conditions being suitable, ample pollination would undoubtedly take place, at even short intervals of favorable weather.

SUNSHINE

Sandsten (*14*) showed that while sunshine had a direct influence upon fertilization in the tomato, it had none in the plum. Judging from his experiments, sunshine appears to have its chief bearing in this connection upon such factors as insect flight and general plant activity, particularly nectar secretion. Kenoyer states (*10*, p. 21) that "clear days are preeminently the days for honey production." From general observation of bee activity on plum bloom, the same may be said regarding pollination. As will be seen later, however, pollen is most readily available for dissemination in dry, sunny weather when bees are most active.

The total hours of sunshine during blooming are less than might be expected. The character of the day is indicated in Plate 15 at the base of the graph for each year by the shading. For the 7-year period there has been, while plums were in bloom, an average of only 49 hours of sunshine each season, compared with an average of 56 hours of cloudiness. The minimum was reached in 1916, when there were only 27 hours of sunshine. Alone, however, the absence of sunshine does not prohibit the setting of fruit.

RAIN

On account of the nature of the processes taking place at blooming time, rain has the most immediate action of all of the factors of weather. The fact that the period of pollination is so limited in the plum makes it possible for rain to delay normal functioning to an injurious extent. Furthermore, the stigma is exposed to weather during the limited time it functions. It will be seen, therefore, that rain may influence processes which, on account of the structure of the organs concerned, must function when more or less exposed.

EFFECT OF RAIN UPON DEHISCENCE

A study of the bloom in the orchard during a heavy and prolonged rain showed that the stamens were drawn together and held in a cluster about the pistil by a large drop of water. This was typically the condition in the absence of wind and in protected locations. The added weight of water held in this way resulted in a drooping of the branches,

[1] Personal correspondence with E. F. Phillips of the U. S. Department of Agriculture, Bureau of Entomology, Dec. 26, 1918.

and a large part of the water dripping from the tree fell immediately from the stamen cluster. When the style was the same length or shorter than the stamens, the stigma was completely immersed in water. In cases where the style was considerably longer than the stamens, the stigma projected from the drop, especially in positions where the pistils pointed upward.

During the period of drying after a rain, when the water holding the stamens and pistils is partly evaporated, the anthers break up into groups, each group, however, being still held in water. Gradually, upon further drying, the groups break up, and the anthers assume their normal position in the flower.

In order to study anther action more in detail at the time of rain, a limb which had been in bloom for three days was cut from a tree during a heavy rain and brought into the laboratory, the temperature of which was about 68° F. All anthers were closed when first brought in, but some

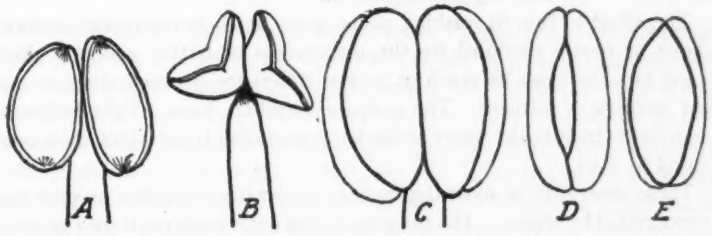

Fig. 1.—An outline drawing of an anther of Minnesota No. 12, showing the adjustment which takes place as a result of taking up or giving off water: A, an anther which has been open in the orchard for three days; B, the same with the anthers pushed up to show the dead area at the upper end of the filament; C, the appearance of the anther after two minutes in water. The anthers are completely closed and have reached their usual size; D, the degree of opening of one suture of the same anthers in 8 minutes when exposed in the laboratory at a temperature of 70° F.; E, the same anther at the end of 12 minutes' drying.

opened completely in 10 minutes under the conditions in the laboratory. When these anthers which had opened were again placed in water they closed in two to three minutes.

Furthermore, anthers which had been open for approximately 3 days and from which all of the pollen had been shed, when placed in water, closed up and in some trials swelled to the usual size in as short a time as 2 minutes (fig. 1). Other tests showed that when unopened anthers were kept in water for 2 weeks there was a slight breaking of tissue at the suture but no dehiscence. On the other hand, anthers which had once dehisced and from which the pollen had been shed closed at once when placed in water and remained closed during the 2 weeks of the test. Opened anthers held for 4 days in a saturated atmosphere under a bell jar did not absorb sufficient moisture to close them; and the experiments of Goff (5) showed that plum anthers did not open in a saturated atmosphere under a bell jar in 56 hours at a temperature of 65° to 70° F. Goff (5) also showed that in a dry atmosphere low temperatures (about 51° F.)

retarded but did not prevent anthers from opening. This shows clearly the relation of dehiscence to water.

The fact that empty anthers close during a rain and open afterwards probably has been the basis for the popular conception that rain washes pollen away.

With this statement, then, of anther action in relation to water, the question arises as to what extent rain removes pollen from anthers which have just dehisced. In investigating this point a branch of flowers was brought into the laboratory, and after the anthers opened it was stirred vigorously for 8 minutes in a pail of water. All anthers closed completely during the time of stirring. The larger part of the pollen lost occurred with the first impact with the water. After this treatment it was estimated that those anthers which were open before being put into the water still contained, when they opened again, from one-quarter to two-thirds of their pollen. These results agree with observations made in the orchard both during and after a rain.

The effect of rain in washing pollen away, even in the quantity noted above, is partly modified by the unevenness of anther opening, there being in some cases as much as 3 days' difference between the first and last opening of anthers. The unopened anthers have a light yellowish color in contrast to the water-soaked appearance of those which have been closed by rain.

These observations show that anther action is a reversible process and is controlled by water. The presence of the anther sap until the maturity of the pollen creates an internal condition unfavorable to dehiscence. If dehiscense takes place only after sufficient drying, there must be an internal control of water as well as a means for external loss. These two conditions are met by a breaking of the epidermis at the suture and by the drying or death of the cells of the filament at the point of union with the anther where there is a pronounced constriction of the filament. At this point the cells typically turn brown before dehiscence, a condition which suggests an early cutting off of water. The browning slowly extends down the filament and at the time the petals fall, 3 to 4 days after blooming, the filament is dead for a distance of 1 to 2 mm.

Under some conditions pollen is shed more quickly than under others. When anthers of Surprise were allowed to open in a dry, still room at about 72° F., at the end of four days pollen had not been shed except in very small amounts. This was due partly to the adhesive action of a yellowish, oily substance about the pollen grains which is characteristic of some varieties, and partly to the absence of wind. The persistence of pollen is further shown by specimens of Surprise grown in the greenhouse, which, at the time of abscission of the calyx tube, 10 days after blooming, still had an abundance of pollen present. But in some varieties with sticky pollen, under orchard conditions as much as one-half may still be present at the time the petals drop. On the other hand,

in some varieties of *P. americana*, pollen may almost completely disappear from the anther during a wind, undoubtedly due to drying and shaking the first day, or even the first few hours after opening. Wind pollination would be more effective in these varieties than in the others, although it is probable that it would be insufficient because plum pollen has no appendages as in *Pinus* spp. to give it greater carrying capacity.

The importance of the rapid closing of anthers upon coming in contact with water, together with the fact that they remain closed as long as they are wet, needs emphasis in this connection. It will be evident that pollination is impossible when the anthers are closed. Furthermore, the conditions which close anthers in most cases also prevent insect flight, but, even if insects were working, pollination could not take place for the reason that pollen is not available. It appears, therefore, that too much emphasis has been placed upon the action of rain in washing pollen away because anthers close quickly enough largely to prevent it.

RAIN INJURY TO PLUM POLLEN

It has been shown above that anthers take up water in sufficient quantities to close them before there is complete loss of pollen. Accompanying the drying process which takes place in the anther and the disappearance of the anther sap, there is a similar drying in the pollen. Before dispersal, pollen changes from the typical spherical shape to one distinctly oblong, and deep folds appear at the sutures. When subjected to drying immediately after removal from the anther, this change in shape takes place in 5 to 10 minutes and is quickly reversible in 3 to 5 minutes when placed in water. With these changes in mind, the question arises as to the effect of a prolonged rain upon pollen.

The rainy period at blooming time in 1915 started with a trace on April 24 and ended with rain all day on April 26 and 27. The heaviest rain, accompanied by a moderate wind, fell on April 26. During the period of the rain there was a relatively high temperature ranging from 58° to 62° F.

Following the usual cytological procedure, before drying, there were fixed in Flemming's medium anthers from 48 hybrids and varieties after the rain of April 26 and from 30 others after the rain of April 27. In all, pollen was collected from 63 crosses and 13 varieties, representing 6 species, namely, *Prunus americana*, *P. Besseyi*, *P. nigra*, *P. triflora*, *P. pissardi*, and *P. cerasus*.

It would appear that this material would furnish conclusive evidence as to whether or not plum pollen is burst by rain, as is held by Hedrick (*8*) and generally by fruit growers. A careful examination of mounted sections from each lot fixed as mentioned above, showed (1) that the pollen was not burst and had every appearance of being normal; (2) that only an occasional anther was devoid of pollen, although most of the sutures were broken; and (*3*) there was no apparent difference in the pollen condition of the different species.

EFFECT OF WATER UPON THE VIABILITY OF PLUM POLLEN

The effect of water on the viability of plum pollen was tested in the sand cherry (*P. Besseyi*). The results are presented in Table II. The time of soaking, 10 minutes, while relatively short, was decided upon because it was thought that if water was injurious at all, it would be desirable to test its effect at the shorter exposure. The time of soaking, however, is much shorter than the actual time the pollen was subjected to water, since it required some time to dry. Sixteen hours elapsed before this pollen was applied to the stigma. It will be seen from these results that soaking pollen of this species in water and drying before using has no injurious effect.

TABLE II.—*Viability test of Sand Cherry (P. Besseyi) pollen after being soaked 10 minutes in water and then allowed to dry for 16 hours, the pollen in one series having been taken from unopened anthers and allowed to dry in the sun and in the other series from open anthers and allowed to dry in the shade*

Cross made and pollen treatment.	Condition of anthers.	Number of flowers on May 25.	Number swelling on June 25.	Number set on June 26.
Treated:				
Tree No. 1×, pollen, soaked 10 minutes	Unopened	9	6	4
Tree No. 1×, pollen, soaked 10 minutes	do	15	6	6
Tree No. 2×, pollen, soaked 10 minutes	do	31	20	19
Tree No. 2×, pollen, soaked 10 minutes	do	26	9	9
Tree No. 3×, pollen, soaked 10 minutes	do	12	.....	4
Tree No. 1×, pollen, soaked 10 minutes	Opened	31	10	9
Tree No. 3×, pollen, soaked 10 minutes	do	28	9	7
Checks:				
Tree No, 1×, pollen, not soaked		7	4	4
Tree No. 2×, pollen, not soaked		19	12	11
Tree No. 1×2, pollen, not treated		.....	21	18
Tree No. 2×1, pollen, not treated		25	11	11
Tree No. 3×4, pollen, not treated		.....	.....	13
Tree No. 3×5, pollen, not treated		.....	.....	11
Tree No. 4×3, pollen, not treated		.....	6	3
Tree No, 5×3, pollen, not treated		.....	6	1

In addition to this, germination tests were made with selected varieties to determine the effect of the rain of April 26 and 27 upon the viability of pollen. Pollen was taken from anthers which had been closed by the rain and placed in a hanging drop of 20 per cent cane-sugar solution. There was no germination even in the checks from tented trees or from unopened anthers subjected to rain. The temperature, however, which was very changeable, was quite low a good part of the time, especially at night, and the negative results with the check make it impossible to draw conclusions as to rain injury to pollen under orchard conditions.

It has been shown that on account of anther adjustment less pollen is actually washed away than has been supposed. Also, considerable quantities of pollen may be retained by anthers which have opened

immediately preceding a rain, owing to the rapidity with which they close. Anthers open as a result of drying, a condition which is brought about by cutting off the water supply at the constriction of the filament, and by evaporation, particularly from the suture. Anthers which have dehisced close quickly when brought in contact with water, and, like those which have not dehisced, remain closed as long as wet. Consequently, pollen is not available for dissemination during a rain. A careful distinction must be made between the normal shedding of pollen, which takes place for the most part the first day or even the first few hours an anther is open, and the washing away of pollen by rain, for the reason that empty anthers close when wet but open again after a rain when dry. Insect visits are reduced to a minimum, if not prevented, under the same conditions that impede pollen dispersal. The cytological studies show that plum pollen does not burst when wet by rain and crossing tests show that it is not killed by moderate exposures to water, although the results of Sandsten (*14*) indicate that humidity decreases its longevity. As far as the pollen is concerned, therefore, a prolonged rain acts primarily to delay pollination until conditions are again restored which are favorable to dehiscence and dissemination.

THE STIGMATIC SURFACE

As in the case of anther and pollen, a study has been made of the changes of the pistil during the functional period, which may be regarded as a critical stage viewed from the standpoint of the relation of adverse weather to the setting of fruit.

Immediately before the receptive period the outer cells of the stigma are turgid (Pl. 14, C and D) and their papillate structure gives to the surface a characteristic velvety appearance which is readily distinguished from the glossy, moist surface when receptive. Where the suture terminates, the stigma has a distinct depression, and in the plum its surface is more or less oblique to the axis of the style, with the higher margin opposite the marginal suture fold.

The terminal cells are one layer thick, and in longitudinal sections are clearly distinct from the cells below on account of their large size, scant cytoplasm, and conspicuous vacuoles. There is a slight variation in the length of these cells in different species. In some, as in Sapa (*P. Besseyi* × *P. triflora*), they contain spherical bodies, which stain deeply and vary much in size, the larger ones being somewhat greater in cross section than the nucleus. The scant cytoplasm in the terminal cells is mostly located at the extreme terminal end in the form of a crescent.

THE RECEPTIVE STIGMA

Decided changes are noticeable in the terminal cells after the stigma has become receptive. In sections made from stigmas 48 hours after first becoming receptive the papillate cells are very irregular in outline

and typically are collapsed and shrunken. A few cell walls appear to be broken. The cytoplasm is much contracted and drawn out into irregular vacuolated strands. The nuclei are generally irregular in outline and show evidence of disintegration. In many of the stigmas the papillate cells are partly broken away from those beneath, and the pollen grains are found among, or even beneath, the collapsed and partly separated sheath composed of the terminal cells.

Heideman states (*9*, p. 191) that the "actual time during which fertilization may be effected scarcely exceeds two hours." Observations here show that under normal conditions the plum stigma remains receptive for a maximum period of about one week. At the end of three to five days, however, the stigma begins to turn brown, and as it becomes dead and dry at the end of the receptive period the color gradually deepens to a dark brown and then black. The dark color slowly extends down the style, which, as a rule, abscisses before turning brown more than two-thirds of the way to the abscission layer. In this way the possible time of pollen-tube growth on the stigma is limited to a relatively short period. The significance of this will be emphasized in connection with the discussion on the rate of tube growth.

THE ACTION OF RAIN UPON THE STIGMA

The prevailing belief among fruit growers is that the chief injury of rain to the stigma, aside from washing pollen from it, is the dilution of the stigmatic fluid to such an extent that the growth of the pollen tube is prevented. Immediately after a heavy rain during full bloom on May 9, 1918, a study of stigmas under orchard conditions showed that even those which were past the receptive stage, dark brown in color and partially dead, were distinctly moist and turgid.

Following these observations an investigation was made of the action of water upon the stigma. When one which had been receptive for about three days was dipped in water and carefully withdrawn, a small droplet about the size of the stigma adhered. This droplet was absorbed in approximately one minute. The dipping was repeated eight consecutive times in as many minutes, and in each case the droplet was as quickly absorbed as in the first instance. As a result of the absorption of water the papillate cells became distinctly turgid. A similar test was made with an unreceptive stigma and also one which had passed the receptive stage and of which the papillate cells had become dark brown and partially dead. The same imbibition of water took place with these two as with the receptive stigma.

It will be evident that absorption of water in such quantities acts to dilute the cell sap of the papillate cells. This, however, would appear to be of no immediate consequence, since pollen does not take up the stigmatic fluid until it is secreted, and even if pollen in this way came

in contact with water before the stigmatic fluid, this, as has been shown, would not be prohibitive of subsequent normal development. Furthermore, since tests show that germination takes place in a considerable range of concentration in a sugar solution, a partial dilution of the stigmatic fluid as a result of water absorption would probably not alone prohibit tube growth. Under greenhouse conditions and in the orchard under favorable conditions a stigma, like a leaf gland, has more than one period of active secretion. If the first fluid to be secreted was completely removed by rain, it would be again renewed under favorable conditions, so that a short rain alone would not necessarily be detrimental. Even if the secretion were considerably diluted following a rain, evaporation from the surface would result in a gradual concentration. Furthermore, the influence of rain upon the stigmatic secretion could be considered of more importance if the stigma had only a single, short period of activity.

WASHING OF POLLEN FROM THE STIGMA

The adherence of pollen to the stigma was first noticed in pistils which had gone through the washing and numerous changes of solution in the preparation for sectioning by the usual cytological procedure. Stigmas which had passed through a 2-day rain, in addition to the cytological process, still held as many as 40 to 50 pollen grains.

An examination under orchard conditions of stigmas which had been subjected to a heavy rain of over 14 hours duration, showed that most of the stigmas still retained a considerable quantity of pollen (Pl. 14, B). On one stigma 42 grains were counted. On another, which had passed through a 2-day rain while in bloom, there were 32 pollen grains, and 6 days afterward on still another there were 176. However, in the last instance a part or all of the pollen could have reached the stigma after the rain.

In order to determine how readily pollen can be washed away, an abundance of pollen was placed on a stigma which was then immersed in water, the results being observed with a binocular miscroscope. At the first impact of the water a few of the outlying grains were washed away, but at the end of 10 minutes of vigorous stirring and dipping in a pail of water, 73 grains still adhered to the stigma. While the number of grains at the start was not counted, it was estimated that less than one-fourth were lost. The outstanding fact is that not all of the pollen was removed by a washing action, certainly as vigorous if not as prolonged as a rain.

An explanation of the adhesion of pollen is found in the condition of the respective stigma. In some of the fixed preparations there is a slight staining area beyond the terminal cells of the stigma (Pl. 14, A and B), in depth about equal to the thickness of two or three pollen grains. This undoubtedly represents the area in cross section of the stigmatic

fluid. Sections of stigmas show, as mentioned above, that during the later stages of receptiveness pollen may be even partly sunken in among the terminal cells. This, together with the gelatinous or viscous nature of the stigmatic fluid, expecially some time after receptiveness, largely accounts for the difficulty in washing pollen from the stigma. Also, the inward movement of water would partly counteract the washing action, especially of light rains. In addition, during the early stages of pollen germination the tubes tend to prevent pollen from being washed away. At this time, however, the tube becomes the important consideration instead of the pollen.

All pistils are not subjected alike to rain action. Those on the upper side of limbs and in terminal positions receive the direct impact of rain, while those in the more protected positions, as in the interior parts of the tree and on the under side of clusters, are shielded from the direct force of the rain.

It will appear from the foregoing that pollen is not so completely washed away by rain as has heretofore been supposed. This belief has become general on account of the changes which take place in pollen when it is placed upon a receptive stigma. Immediately upon coming in contact with the stigmatic fluid, pollen becomes turgid and is more or less immersed in it. Under these conditions its appearance closely resembles that of the terminal cells of the stigma. If a dilution of the stigmatic fluid and the washing away of pollen are the important inhibiting factors in the setting of fruit, a short dashing rain at blooming time would, at certain stages, do as much damage as a prolonged rain, because it would be necessary for the pistil to again become receptive and pollination to again take place. This, however, does not correspond with the general observations of fruit growers nor with the conditions reported here.

LIMITATIONS UPON FERTILIZATION

If the statements regarding the effect of rain upon pollen and stigma are correct, the failure of the plum to set fruit during unfavorable weather conditions will have to be explained in another way. At the time the pollen and pistil are maturing and functioning other factors are operating which place certain definite limits upon the time fertilization is possible.

On account of self-sterility, the relative time of dehiscence and receptiveness within the variety is not an important factor in the plum. However, because the pollen is mature before the stigma and virtually in a "resting stage" protected by a thick covering in addition to the anther wall, it is less susceptible to injury than the stigma, in which growth changes are still taking place. This difference in the relative maturity of the two structures may largely account for the greater hardiness of pollen during frosts. Upon germination the pollen enters a phase of less resistance, and it shares to a greater extent the lot of the stigma and style, which constitute the substratum for the pollen tube.

The factors, then, which place a time limit upon the mutual functional period and which have a direct bearing upon the setting of fruit are (1) the longevity of the pollen, (2) the length of the receptive period and life of the stigma, (3) the abscission of the style, (4) the rate of the pollen-tube growth, and (5) the influence of low temperature upon pollen germination.

THE LONGEVITY OF PLUM POLLEN

The results of Sandsten (*14*) showed that plum pollen collected from such widely separated sources as Washington, Missouri, Tennessee, and Minnesota retained its germinating power for six months when subjected to the normal humidity and temperature changes incident to the period of the test. There was a gradual decline, however, in the percentage of germination from an average of 54 per cent at the end of the first month to about 8 per cent at the end of the sixth. Furthermore, relatively adverse conditions do not affect the longevity of the pollen, since short exposures to water do not kill it and freezing temperatures only retard germination. Under favorable conditions, therefore plum pollen retains its viability considerably longer than it is functional under orchard conditions.

LENGTH OF RECEPTIVE PERIOD AND LIFE OF THE STIGMA

As has been noted, the plum stigma is receptive under orchard conditions for a maximum of one week but begins to turn brown at the end of approximately three to five days. Adverse weather conditions may, however, extend the functional period somewhat, particularly when accompanied by low temperatures. The delay in pollination up to a certain point does not prevent tube growth. Crosses were successful in the greenhouse on stigmas which were receptive four days previous to the application of pollen. Under these conditions, however, drying and browning does not take place as quickly as in the orchard where the active period of secretion is over at the end of three to five days and is followed by a period of partial inactivity of the stigma.

Furthermore, the stigma is more easily dried by the wind late in the receptive stage than immediately after becoming receptive. Tube formation would undoubtedly be more uncertain if pollination were delayed until late in the receptive period, as would be the case during a prolonged rain. Pollen germination, as well as considerable tube growth, must, therefore, take place if fertilization is to be effected within a relatively short time and before the conditions of the stigma prohibit tube growth or before dying back in the style overtakes tubes which have been formed.

ABSCISSION OF THE STYLE

The styles do not begin to absciss until about two weeks after blooming (Pl. 14, E), although the abscission layer at the point of abscission near the

base becomes very distinct in some varieties, as Winnipeg (*P. nigra*), as early as 8 days after blooming. In this variety at the 8-day period the cells in the abscission layer had reached an advanced stage in their disintegration, and while the style was still persistent, it was much lighter in color above the point of abscission, a condition which suggests the cutting off of food material. If the pollen tube had not passed the abscission layer by this time, it is probable that it would not have done so, since it would have had to grow through a region of partly disintegrated cells. Consequently all tubes which had not passed the abscission layer by the time of the abscission of the style (Pl. 14, F) would be definitely eliminated as far as fertilization is concerned. Tube growth from the stigma to the abscission layer, therefore, must take place between the beginning of receptiveness and the shedding of the style.

If pollination occurs late in the receptive period, the condition of the stigma begins to change so rapidly that only favorable growing conditions for the tubes will enable them to pass the abscission layer before the style drops. In this way the abscission of the style sets a definite time limit to a certain minimum of tube growth which may be as short as 4 days and as long as 12. It will be clear then that the later in the receptive period pollination takes place and the more tube growth is retarded, the more uncertain fertilization becomes.

RATE OF POLLEN-TUBE GROWTH

It will be seen from the above that the rate of pollen-tube growth becomes an important factor in fertilization, especially during unfavorable weather accompanied by rain and low temperatures. In order to determine the rate pollen tubes advance down the style, this point has been studied in fixed preparations of pistils taken under orchard conditions and also from controlled crosses in the greenhouse where the time of pollination could be determined definitely. The greenhouse temperature during this experiment was not recorded, but varied from 55° to 65° F. Pistils from the orchard in all cases were collected after a period of variable weather of rain and low temperatures. The results showing the extent of tube growth under different conditions are presented in Table III.

Sandsten (*14*), in determining the time required for the pollen tube to reach the ovary, cut the pistils off controlled crosses at intervals of 48 and 60 hours, respectively. From the data he presented it appears that the plum is fertilized at the 60-hour period. It should be stated, however, that this shows that tube growth had merely extended below the point at which the style was cut in that time. The 7-day period, at which time the final observations were made, is too soon to determine certainly whether fertilization has taken place judging from size alone.

TABLE III.—*Rate of pollen-tube growth found in the plum in controlled crosses in the greenhouse and under orchard conditions*

UNDER GREENHOUSE CONDITIONS

Cross.	Time.	Tube growth in greenhouse.
Minn. No. 10 [a]×Minn. No. 12.[a]	16 hours	1/6 of style length. Cross sterile.
Minn. No. 10 [a]×*P. Besseyi*	do	½ of style length.
Minn. No. 12 [a]×Minn. No. 21.[a]	9:30 a. m. to 3:30 p. m.	None.
Do	17.5 hours	1/10 of style length.
Minn. No. 12 [a]×*P. Besseyi*	19 hours	¼ of style length.
Minn. No. 21 [a]×Minn. No. 12.[a]	51 hours	Do.
Minn. No. 21 [a]×Minn. No. 10.[a]	69 hours	1/10 of style length.
Minn. No. 6 [a]×Surprise	6 days	Full style length. None fertilized.

UNDER ORCHARD CONDITIONS.

Minn. No. 21 [a]×open-pollinated.	3 days after blooming	No tube growth. Rain and frost.
Minn. No. 35 [b]×open-pollinated.	do	Do.
Do	6 days after blooming	¼ of style length.
Minn. No. 12 [a]×open-pollinated.	do	Do.
Do	10 days after blooming	Tube in embryo sac.
Minn. No. 12,[a] selfed	4¼ hours	Tube just formed.
Do.[c]	24 hours	1/10 of style length.
Minn. No. 6,[a] selfed	2 days	Do.
Manitoba, selfed	8 days	¼ of style length.
P. Besseyi, selfed	12 days	⅔ of style length. Ovule aborted.
Surprise, selfed	6 days	1/10 of style length.

[a] A cross between Burbank and Wolf. [b] A cross between Abundance and Wolf. [c] See Pl. 14, A.

From Table III it appears that pollen-tube growth is relatively slow in the plum and that the time required for the tubes to reach the ovary is much longer than Sandsten estimated. Furthermore, it should be emphasized that in the above table the maximum tube growth is given.

It will be seen in the case of Minnesota No. 21 and No. 35 that there was no tube growth three days after blooming when open-pollinated under orchard conditions. The weather conditions previous to the time stigmas were collected from these two varieties will be of interest here. Both came into bloom on May 20, 1917, which was clear, with a maximum temperature of 62° F., with a slight rain in the evening, and a medium wind the latter part of the day. At night the temperature fell and there was frost. May 21 was cloudy, with a heavy rain accompanied by a strong wind lasting from early morning up to 2 p. m. May 22 was cool and clear, and the stigmas of these two varieties were collected in the early forenoon.

The time of pollination is uncertain, but bees were present in large numbers on May 20. On a single stigma of Minnesota No. 35 there were 162 pollen grains, mostly embedded in the stigmatic fluid. There were fewer grains on the stigmas of Minnesota No. 21. In the field records, made at the time of fixing this material, it was stated that the "stigmas were brown in all cases and dead in some." From this it will be seen that the receptive period was much shorter than is common in the plum. The condition, then, in these two varieties was as follows: (1) Pollination had taken place, (2) on the third day after bloom no tubes had formed in the stigmas examined, and (3) the end of the receptive period had been reached.

On each variety the dying back in the styles averaged 5 mm. by May 31, and by June 2, 13 days after bloom, the abscission layer was fully formed and disintegration of the cells in it had started. On this date additional pistils were collected and fixed, and in these pollen tubes could not be found in the micropyle, nor had embryos formed in any of the six which were sectioned. This is not surprising when it is noted that under the favorable conditions of the greenhouse, Surprise pollen tubes required six days to grow the full length of the style.

These trees under observation were 8 years old from planting and were under clean cultivation. On Minnesota No. 21, 25 per cent of the buds were winterkilled and only 5 per cent of the flowers set fruit; on Minnesota No. 35, 10 per cent were winterkilled and the percentage of fruit to set was 10. On each there was a light crop of ripe fruit.

In the case of these two varieties, then, the small percentage of fruit to set is not necessarily due to a lack of pollination, but apparently to the delay in tube formation, during which the stigmas turned brown and some died, conditions which either prevented or delayed tube growth. According to this, in those fruits which set, tube growth had either started on the 20th, before the rain, or was sufficiently rapid after it to pass the abscission layer before the style fell. The weather conditions for this season are analyzed in Plate 15.

From Table III it will be further seen that under the favorable growing conditions of the greenhouse, the rate of tube growth is so slow that the abscission layer is passed dangerously near the time of dehiscence. In the orchard, however, during the most suitable conditions, fully as many fruits set as in the greenhouse, and it is very probable that the tube extension is even more rapid.

The bearing of low temperatures upon the status of tube growth noted above warrants further discussion. The lower temperature limit of pollen germination in the plum was determined by Goff (5), as previously noted, to be approximately 40° F. At 70° F. there was an abundance of tube growth, and at 51° F. the rate of growth was intermediate between the two extremes. Entering the factor of humidity in relation to temperature, his experiments further show that there was

greater germination after five days, when pollen was kept in saturated air in a refrigerator (the temperature is not given), than under the same conditions at room temperature. This being the case, the cooler temperatures usually accompanying prolonged rains would be more favorable to a higher percentage of germination than higher temperatures. From Plate 15 it will be seen that each season the minimum temperature falls below the lower limit of tube growth a number of times and occasionally the lower limit of tube growth is even approached by the maximum temperature. It is probable that the temperature influence on tube growth would be similar to that on tube formation.

The slow pollen-tube extension found under greenhouse conditions serves as a basis for estimating what can be expected during periods of low spring temperatures. That low temperatures have a much greater influence some seasons than others is clearly shown by the extent the minimum-temperature curve extends below the line of no tube growth (5) drawn through each graph (Plate 15) at 40° F. The temperature factor, therefore, has an important bearing upon the extent to which fertilization fails to take place some seasons. While cool weather retards tube growth, it does not appear to change materially the time of abscission of the style.

RELATION BETWEEN THE WEATHER AT BLOOMING AND THE SETTING OF FRUIT

With the foregoing analysis of weather in mind, it now remains to be seen whether there is any correlation between the weather conditions prevalent at bloom and the setting of fruit. While an ample set of fruit does not certainly insure a full crop, a full crop can not be obtained unless there is a set up to a certain point. In this way the weather determines the possibility of a crop.

During the years 1915, 1916, and 1917 there was a light set and a light crop of plums at the Fruit-Breeding Farm. An inspection of Plate 15 shows that different weather combinations occurred during each of the three years. In 1915, the outstanding features are the frequent rains during bloom and the low-temperature period for one week following. This single factor, according to the work of Goff (5) on the temperature limits of tube growth, would make fertilization uncertain, but it will be noted that following the cloudy, rainy weather of the first four days of bloom there were two days of unusually windy weather which interfered with bee flight at a critical time, and hence rendered ample pollination uncertain. The following year, 1916, bloom was nearly a month later and was accompanied by a period of unusually high temperature which extended to the period of tube growth. This alone would have been very favorable to pollination, but during early bloom there were two unusually heavy rains and five lighter ones. Moreover, aside from actual injury to the bloom during such rains as

occurred on May 21 and 25, as well as the interference with insect flight, pollination would appear to be uncertain because pollen was not available for dissemination a large part of the time. This year, therefore, it appears that pollination was uncertain instead of fertilization, as was the case the year before. At any rate, during these two seasons the temperature at bloom was very different. In 1917 rain, high winds, low temperatures, and even frost, were prevalent during bloom, and at the close of bloom there were nearly 3 days of cool, rainy weather which came at a critical time during tube growth. In addition to this, frequent rains and a relatively low temperature at the latter part of the 10-day period following bloom supplemented the retarding effect of the 3-day rainy period. The wind on May 20, 21, 22, and 26 was strong enough to interfere with the work of bees. Both pollination and fertilization were uncertain this year.

In contrast to the slight set of these three seasons there was a good set in 1912, 1913, and 1918, and a heavy set in 1914. It now remains to be seen whether there were conditions at bloom these seasons which differ markedly, as far as the influence on pollination and fertilization is concerned, from the others. In 1912 the temperature was relatively high, except for three days, during the entire period. The rains were slight at bloom. Also, in 1913 the temperature was within the range of fast tube growth a good part of the time and rains were unusually scant at bloom. The unusually high temperature in 1914 is in marked contrast to the low temperature the following year, and in the absence of heavy rains there was the greatest setting of fruit as well as the heaviest crop of all season included. The high temperature at the beginning of bloom in 1918 gradually fell toward the end and there was a frost the night of May 12. The rains were not prolonged during bloom, but the heavy rain of May 9 delayed pollination in the later blooming varieties. The warm period following bloom, however, counterbalances the cooler 4-day period at the end of bloom, so that the rate of tube growth was in general increased. The setting of fruit was sufficient for a good crop this season.

It will be seen from this brief analysis that there are conditions each season which can be correlated with the set of fruit. With a light set it is impossible to get a heavy crop. As early as the 5- or 6-week period the possibilities of a crop are determined.

SUMMARY

(1) Unfavorable weather at blooming time may completely prevent the setting of fruit in the plum, even though there be a full bloom. A study of the manner in which weather affects the processes at bloom shows that rain and low temperatures are the most important factors, although strong winds when prolonged are also important.

(2) Wind has its influence indirectly by interfering with insect action and, hence, pollination at critical times. It is seldom strong enough to cause much direct mechanical injury. The experiments of Waugh show that wind pollination is insufficient, even under the most favorable conditions. Frosts during bloom are only occasional and injure the pistil more than pollen. The greatest damage from low temperatures is in the retarding of pollen-tube growth. Other conditions being favorable, cloudiness does not prevent the setting of fruit. Rain prevents pollen dissemination by closing the anthers or by preventing them from opening, but does not burst pollen nor kill it.

(3) On account of the adhesive action between stigma and pollen, rain does not completely wash pollen from stigmas. The stigma is receptive for 4 to 6 days, and following the active period of secretion the stigmatic cells rapidly disintegrate. The style abscisses in 8 to 12 days after bloom. Tube growth appears to be relatively slow in the plum even under favorable greenhouse temperatures. As a result of the rapid disintegration in the stigma and the abscission of the style, a delay in pollination or slow tube growth when the temperature is low renders fertilization uncertain.

(4) An analysis of the prevailing weather at bloom shows that each season certain sets of conditions can be singled out as being largely responsible for the status of the setting of fruit. In one season rain during bloom may be the limiting factor and in another low temperature during the period of tube growth. Unfortunately, practical remedies under orchard conditions do not appear readily available. Late blooming has not escaped unfavorable weather, and, since tube growth seems to be the process most directly affected by low temperatures, remedial measures can most effectively be sought in suitable pollinizers which show the fastest tube growth.

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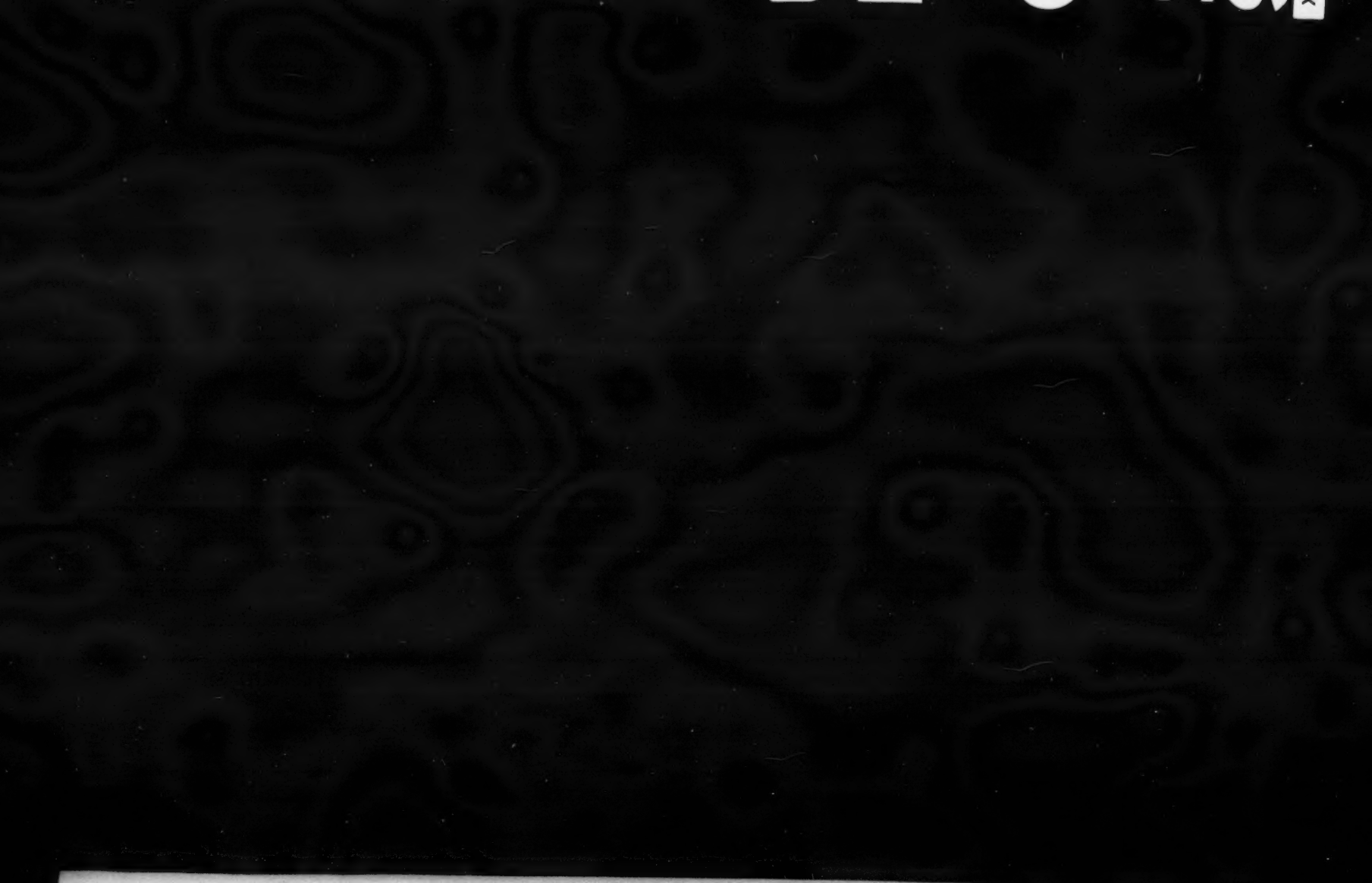

PLATE 13.

Plum tree and fruiting branch showing difference between number of flowers borne and quantity of fruit set:

A.—The appearance of a plum tree bearing a normal crop of bloom.

B.—A single fruiting branch 2 years old showing the contrast to A. Only 2 fruits have set out of approximately 100 flowers borne by this branch. Note the stubs where flowers have dehisced.

A
B

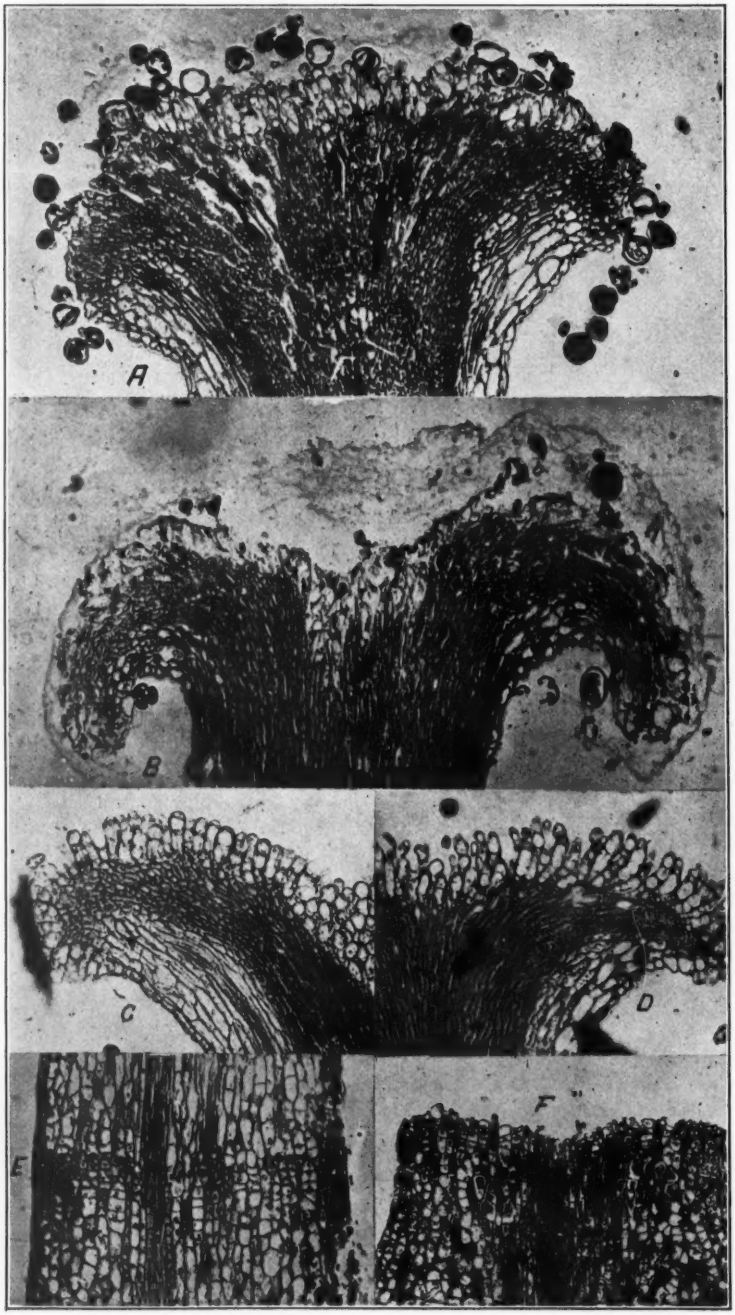
A
B
C
D
E
F

PLATE 14.

A.—Stigma of Minnesota No. 21, a greenhouse tree, 24 hours after being selfed, showing the condition of papillate cells in the stigma, pollen tubes, and also traces of the stigmatic fluid.

B.—Stigma of Minnesota No. 35, open to cross pollination, showing the condition of a stigma three days after bloom, having withstood a rain of 0.87 inch which fell in the two days previous, lasting in all 18 hours. Note the slight staining area of the stigmatic fluid in which two pollen grains are embedded.

C.—The turgid papillate cells in Sapa before receptiveness.

D.—Opata. Same as C. Pollination has not yet taken place.

E.—Abscission layer Minnesota No. 35, showing the cells of the layer 11 days after bloom.

F.—The surface at the abscission layer of Assiniboin after the style has fallen, 12 days after bloom. There appears to be no marked disintegration of the cells immediately below the abscission layer, which suggests that in cutting off the style by this method the breaking down of the middle lamella is restricted to a few cell layers.

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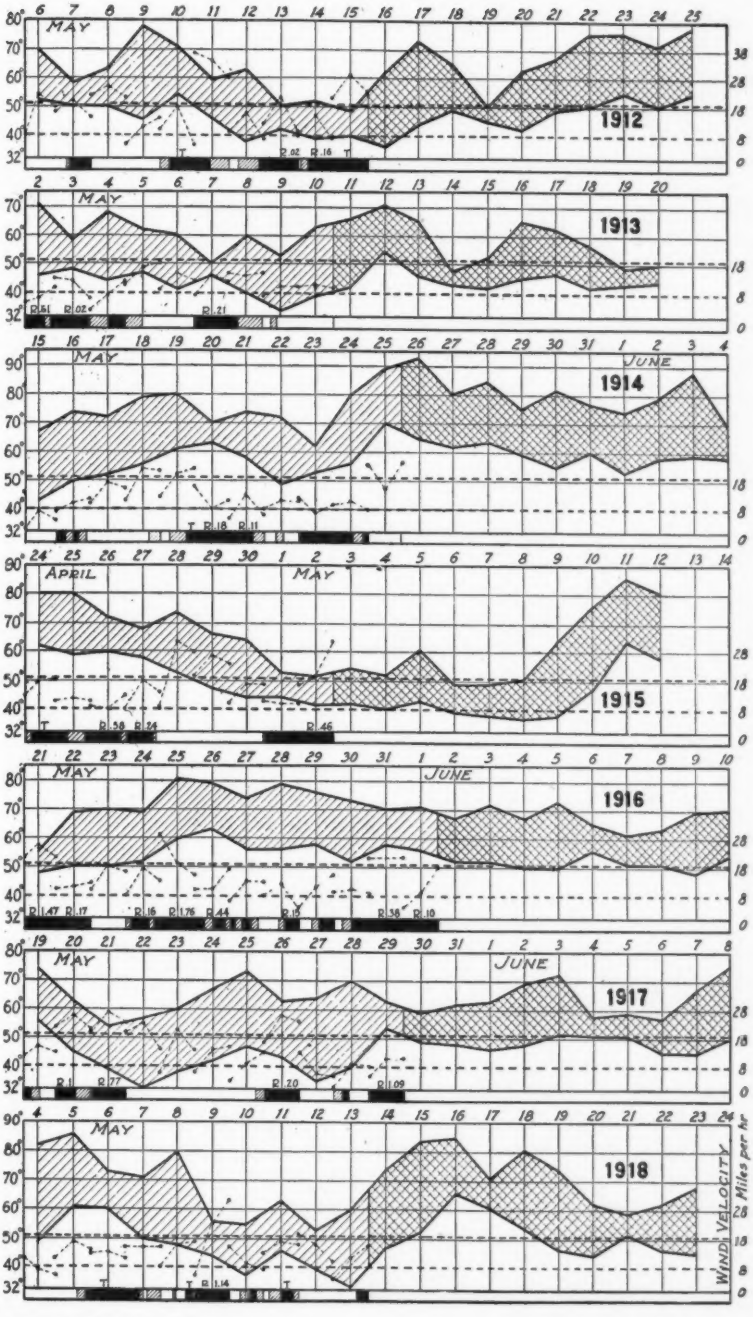
PLATE 15

Graphic analysis of the weather from the standpoint of wind, sunshine, rain, and temperature for seven years from 1912 to 1918. The maximum and minimum temperature range is given for each day during bloom and for a period of 10 days afterwards.


MAY
1912
R.02 R.16 T
MAY
1913
R.61 R.02 R.21
MAY
JUNE
1914
T R.18 R.11
APRIL
MAY
1915
T R.58 R.24 R.46
MAY
JUNE
1916
R.47 R.17 R.16 R.176 R.44 R.38 R.10
MAY
JUNE
1917
R.1 R.77 R.20 R.1.09
MAY
1918
T R.1.14 T
WIND VELOCITY Miles per hr

STRUCTURE OF THE MAIZE EAR AS INDICATED IN ZEA-EUCHLAENA HYBRIDS

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INTRODUCTION

In attempting to trace the origin of maize (*Zea mays* L.) the center of interest is the evolution of the peculiar form of inflorescence, especially the pistillate inflorescence, or ear.

Since Euchlaena (*Euchlaena mexicana* Schrad.) or teosinte, the nearest known relative of maize, has a very different type of pistillate inflorescence, it may be instructive to compare the two genera and trace the successive changes that would be required in passing from the Euchlaena form of pistillate inflorescence to the maize ear.

Euchlaena and maize cross freely, resulting in intermediate hybrids which in subsequent generations grade back to the parental forms (Pl. 16). It is therefore possible to present a complete series of intermediates, graduated to any desired degree of minuteness. It should be kept in mind that although we may be able to arrange a continuous series of forms ranging from Euchlaena to maize, these forms may not represent the course of evolution. A study of these intermediate hybrids may be expected, however, to throw light on the morphology of the ear and to explain its evolution, at least in a mechanical sense.

DESCRIPTION OF MATERIAL

The forms here described as intermediate between maize and Euchlaena appeared for the most part among the descendants of a cross between Florida teosinte and a diminutive variety of popcorn called "Tom Thumb." Of this cross six first-generation plants were grown and from the self-fed seed of one of these a second generation consisting of 127 plants was produced. Several hundred third-generation plants from open pollinated seed were also examined.

Although in general appearance the pistillate inflorescences of maize and Euchlaena are so unlike that comparisons are difficult, the structure of the flowers is practically identical. The chief differences are therefore to be sought in the structure of the inflorescence and the arrangement of the spikelets.

To avoid circumlocution it is necessary to consider as a morphological unit the association represented by a sessile and pedicelled spikelet, as they occur in the staminate inflorescence. It would be misleading to refer to this unit as a pair of spikelets, because the same unit must also be kept in mind in considering the pistillate inflorescence where one of the spikelets may be suppressed. The two spikelets of a pair probably arise from a single metamer, at least they seem never to become separated. In the pistillate inflorescence, however, the individual metamers can be distinguished with difficulty and the pairs of spikelets become so profoundly and diversely modified that a general term is needed to designate this structural unit in all its forms.

In the pistillate inflorescence the members of this morphological unit, whether it is represented by one or two spikelets, occupy a single alveolus, and the complex might be described as the contents of an alveolus. In the staminate inflorescence, however, the depression in which the spikelets are borne is usually too slight to be termed an alveolus. It seems desirable, therefore, to derive the general term from some word that carries the same implication as alveolus but which has not been used in a specific morphological sense. The word *alicole* [1] is proposed and will be used in the following description to designate the spikelet or spikelets, whether staminate or pistillate, that are borne in a single alveolus or at a single point on the rachis, considered as the axil or point of attachment of a reduced branch.

The principal differences between the pistillate inflorescences of Zea and Euchlaena may now be contrasted as follows:

Euchlaena	*Zea*
Single spikelets	Paired spikelets
Two-ranked alicoles	Many-ranked alicoles
Separate alicoles	Yoked alicoles

SINGLE AND PAIRED SPIKELETS

The difference between single and paired spikelets will be best understood by considering first the arrangement of the spikelets in the staminate inflorescence of Euchlaena, which is identical with that of the lateral branches of the staminate inflorescence of maize. Since Euchlaena lacks the specialized central spike of the maize tassel it may be taken to represent the primitive arrangement of the spikelets.

In these staminate inflorescences each alicole consists of two spikelets—one sessile, the other pedicelled. The alicoles are disposed on the two sides of the branch, leaving the lower, and, to a less extent, the upper side of the branch, naked. The sessile spikelet is borne slightly below the pedicelled, that is, toward the abaxial side of the branch. Thus when viewed from the end of the branch the arrangement of the spikelets would be such that instead of an alternation between pedicelled and

[1] *Ala*, armpit + *colo*, inhabit.

sessile spikelets, the two sessile spikelets would stand next each other as would the two pedicelled spikelets (see fig. 1, A). This lack of radial symmetry will be shown to be a very persistent and important feature.

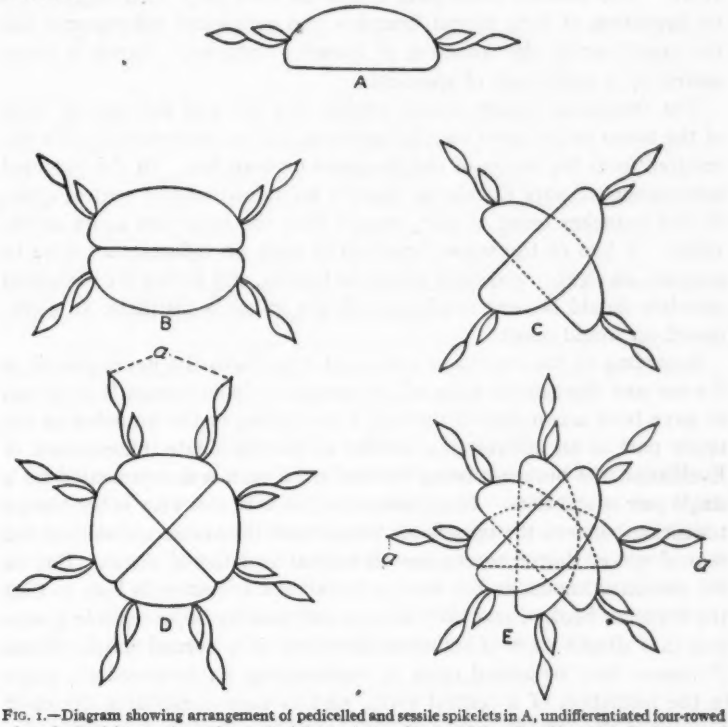


FIG. 1.—Diagram showing arrangement of pedicelled and sessile spikelets in A, undifferentiated four-rowed branch; B, eight-rowed ear, the result of the fasciation of two undifferentiated branches; C, eight-rowed ear the result of twisting a single undifferentiated branch; D, 16-rowed ear, the result of fasciation; E, 16-rowed ear, the result of a further twisting of "C."

Turning now to the pistillate inflorescences a striking contrast appears. Both spikelets are sessile in Zea, and it is usually impossible to determine which of the pair is the homologue of the pedicelled spikelet. In the pistillate inflorescence of Euchlaena, the spikelets are borne singly instead of being paired. It is the pedicelled spikelet that is suppressed, as is clearly shown in the hybrid plants where all stages of suppression can be observed (Pl. 17, A). Furthermore, in pure Euchlaena staminate branches frequently have pistillate spikelets at the base. In such specimens at the place where the transition occurs, rudiments of a pedicelled staminate spikelet can often be seen by the side of a sessile spikelet bearing a well-developed seed.

TWO-RANKED AND MANY-RANKED ALICOLES

The manner by which the number of rows has been increased in the pistillate inflorescence of maize has been the subject of much controversy. Two ways of developing additional rows have been suggested—by fasciation of long lateral branches of a compound inflorescence like the tassel,[1] or by the reduction of branches until each branch is represented by a single pair of spikelets.

The fasciation theory would explain the ear and the central spike of the tassel in the same way, by assuming that a many-rowed spike has resulted from the fusion of simple spikes or branches. In the terminal inflorescence of pure Euchlaena there is no indication of a central spike, all the branches being similar, except that the lower are again subdivided. If two of the upper branches of such an inflorescence were to coalesce, an eight-rowed spike would be formed, and if then the pedicelled spikelets should become sessile and all the spikelets pistillate, an eight-rowed ear would result.

According to the second or reduction hypothesis the development of the ear and the central spike of the staminate inflorescence is supposed to have been accomplished through a shortening of the branches in the upper part of an inflorescence similar to the staminate inflorescence of Euchlaena, the branches being reduced until each was represented by a single pair of spikelets. In apparent conflict with this view is the abrupt transition between the uppermost branch and the lowest spikelets of the central spike, that characterizes all normal varieties of maize. But in the mutation known as *Zea ramosa* the abrupt transition is lost, so that the branches become gradually shorter and pass by imperceptible gradation into simple pairs of spikelets like those of a normal tassel. Thus, *Z. ramosa* may be looked upon as representing an intermediate stage in the formation of a central spike, and as such constitutes the chief support of the reduction theory.

The evidence derived from hybrids of maize and Euchlaena does not support either of these theories. On the contrary, the hybrid plants provide an unbroken series of stages connecting the Euchlaena spike with the maize ear that clearly indicates a third method of increasing the number of rows and forming a central spike or ear. This is by shortening and twisting the rachis of a single spike of Euchlaena, accompanied by an increase in the number of alicoles. The stages in this process will be discussed in more detail later.

SEPARATE AND YOKED ALICOLES

In the pistillate inflorescence of pure Euchlaena the joints of the rachis, each of which bears a single alicole, stand almost directly above one another, resembling a string of triangular beads. One of the most

[1] The earliest published statement of the fasciation theory that has thus far come to light is an anonymous account (Sexual flowers in Indian corn), in Meehan's Monthly, v. 3, p. 105, 1893.

frequent and obvious indications of admixture with maize is a shortening of the rachis. The reduction in length, however, is not uniform but is more pronounced in alternate internodes, with the result that the alicoles become associated and yoked in pairs, the members of which stand nearly opposite to each other.

In the staminate inflorescence of either Euchlaena or the common varieties of maize there is little indication of this yoking of the alicoles. The pairs of spikelets stand on opposite sides of the rachis, but usually they are equally spaced with no indication of yoking, this tendency not even appearing in the pistillate inflorescence of the first generation of the hybrid between maize and Euchlaena. Yoking of the alicoles is, however, a striking characteristic of the second generation and appears in all the stages between the four-rowed spike and a well-formed ear. With the increase in the number of ranks of alicoles this yoking of the alicoles into pairs is obscured, but there are evidences that it still persists even in the fully developed many-rowed ear.

In addition to the sharply contrasted characters discussed above, the pistillate inflorescence of maize differs from that of Euchlaena in having the alicoles much more numerous and more closely crowded.

EUCHLAENA × MAIZE HYBRIDS

Having outlined the nature of the differences between the pistillate inflorescences of Zea and Euchlaena, the pistillate inflorescences of the hybrid plants may now be examined. In the first generation the spikelets are paired, the alicoles separate, and two-ranked. In number of alicoles and degree of crowding they are intermediate between the parents. This mixture of characters derived from both parents creates the general impression that the inflorescence is intermediate.

SECOND AND LATER GENERATIONS

Treating the three contrasted characters of maize and Euchlaena as alternative, there are eight possible combinations: (1) Spikelets single, alicoles separate and two-ranked; (2) spikelets single, alicoles separate and many-ranked; (3) spikelets single, alicoles yoked and two-ranked; (4) spikelets single, alicoles yoked and many-ranked; (5) spikelets paired, alicoles separate and two-ranked; (6) spikelets paired, alicoles separate and many-ranked; (7) spikelets paired, alicoles yoked and two-ranked; and (8) spikelets paired, alicoles yoked and many-ranked. With the exception of No. 6, all of these combinations have been found in second-generation plants and most of them in the descendants of a single cross. To class the individuals into the above eight combinations is, however, a very inadequate expression of the diversity. The dominance shown in the first generation was not followed by any clear-cut segregation in the second. On the contrary, a complete series of intermediates connected the parental forms with respect to each of the three contrasted pairs of characters.

TRANSITION FROM A TWO-ROWED SPIKE TO A MANY-ROWED EAR

The pistillate inflorescence of Euchlaena may be looked upon as a two-rowed ear. In hybrids between maize and Euchlaena the initial step from such a two-rowed ear to one with four rows may be made in two quite different ways. The more common method is for the pedicelled spikelets, which are suppressed in Euchlaena, to reappear. This converts the flat two-rowed spike into a flat four-rowed spike, the condition that obtains in the first generation of the hybrid (Pl. 17, B).

In some instances, however, another method is followed. Alternate internodes of the spike become shortened until the alicoles, each with a single spikelet, are yoked in pairs, the members of which stand opposite or nearly so. The rachis then twists until each pair of alicoles, instead of standing over the one below, stands at right angles with the pair immediately above and below (Pl. 16, D). This results in a square four-row ear. The pairs of alicoles are crossed and fitted into each other in a way that has suggested the name "saddleback" for this type of spike with four rows of alicoles.

In some instances still another step is taken before the spikelets are doubled in the alicole. The rachis is still further shortened and twisted, resulting in a six-rowed ear. Six-rowed ears are sometimes found in which both sessile and pedicelled spikelets are developed. In such cases it appears that the definite relation which ordinarily exists between yoked alicoles has been lost, and starting with the flat four-rowed ear every third alicole has slipped around so that it occupies a plane between the other two, which in turn are slightly displaced (Pl. 17, C).

Returning now to the more common form of a four-rowed ear, it is to be noted that the spike is four-rowed and the pedicels have been shortened, though the distinction between sessile and pedicelled spikelets can still be made out with certainty. The rachis also has been shortened and forced into a series of sharp angles and as a result of such crowding it has now begun to twist (Pl. 17, A).

The next clearly marked stage is the eight-rowed ear. The shortening of the rachis has continued, with increased crowding and twisting of the axil, forcing the alicoles, each bearing a pair of spikelets, to slip past one another into the unoccupied spaces of what were the upper and lower sides of the original horizontal branch. This is again a saddleback type, with the alicoles associated as in the square four-rowed ear described above, though each alicole contains two spikelets instead of one (Pl. 15, F). Intermediate stages between the flat four-rowed ear and the eight-rowed saddleback stage can sometimes be found where the twist is not quite a quarter turn, but all such appear to be unstable. The saddleback, on the contrary, is stable and will sometimes be shown consistently throughout a plant of the second generation (Pl. 18).

A further shortening of the rachis brings about the next stage, which is that of a 10-rowed ear. Intermediate stages are more common during the acquisition of this stage, and when they occur the seeds, as might be expected, are not arranged in regular rows.

With these facts in mind, the spike can be understood as composed of opposite or yoked alicoles, each with a pair of spikelets. These yokes are superposed, and as crowding increases there results a further twisting and the formation of a more complicated spiral. With seeds of a uniform size a compact spiral would result in the formation of longitudinal rows, though these might not run exactly parallel to the axis of the ear, as, indeed, they seldom do even in ears of maize.

MORPHOLOGY OF THE MAIZE EAR

It has been shown that the intermediate forms that appear in hybrids between maize and Euchlaena afford no support for the fasciation theory. Evidence from the ear of pure maize may now be presented.

If a number of four-rowed branches were forced together and their axes united, the conditions found in an ear of maize might result (see fig. 1, B). There is, however, evidence in the ear itself that it is not constructed in this way.

It is not an uncommon occurrence for an ear to drop rows. For example, there may be 12 rows at the base and only 10 rows at the tip. A study of how this transition is made throws light on the morphology of the ear. In the first place, the loss is almost invariably two rows, and both are lost at the same distance from the butt of the ear. There is no region with an odd number of rows. A normal ear is made up of a series of paired rows and this is usually accepted as an adequate explanation of the fact that the number of rows is always even. A pair of rows is looked upon as the fundamental structural unit of the ear, a view in accord with the theory of fasciation. Since two rows are dropped at once, it might be expected that the interrupted rows would be adjacent. This would follow from the suppression of a pair of rows representing the sessile and pedicelled spikelets arising from a single series of alicoles.

There is, however, abundant evidence to show that rows are usually interrupted by the abortion of pedicelled spikelets only. This can be seen in abnormal maize tassels in which the base of the central spike is pistillate, forming in reality a section of an ear. At the place where the transition occurs it can be seen that the sessile spikelets are more persistent and produce larger seeds.[1]

[1] With the idea of determining to what extent differentiation between pedicelled and sessile spikelets persists in the fully developed maize ear, the weight of each of the two seeds from individual alicoles was compared. An ear of flint corn was chosen in which the alicoles were clearly marked and the individual seeds were carefully weighed. There were 135 alicoles with two comparable seeds. The average weight of the individual seeds for all the seeds was 430 mgm. The average difference between the seeds of an alicole was 21.0 mgm. ± 19.5.

It would appear, therefore, that if there was any consistent difference between the weight of the seeds borne in pedicelled and sessile spikelets in this ear, the difference must have been something less than 5 per cent of the weight of the seed.

With the recognition of the fact that the interrupted rows represent pedicelled spikelets instead of the pedicelled and sessile spikelets of a row of alicoles the position of the interrupted rows with respect to one another becomes of importance in studying the formation of the ear.

Following the fasciation theory, if both of the rows of pedicelled spikelets of a single branch aborted leaving the sessile, we should find the two interrupted rows separated by two remaining rows. (This may be illustrated by reference to fig. 1, D. If the two rows of pedicelled spikelets marked *a* were aborted the two missing rows would be separated by two rows.) This is not what occurs. In the examination of many ears in which rows were dropped no instance has been found where the dropped rows were either adjacent or separated by two rows. In cases where the location of the dropped rows can be determined with reasonable certainty the dropped rows are on opposite sides of the ear. Yet they are not exactly opposite, but missing it by just two rows. This is what should occur if the two pedicelled spikelets were dropped simultaneously from a pair of yoked alicoles. It will be recalled that the dorsoventral arrangement of the spikelets in the original four-rowed spike results in bringing the pedicelled spikelets not exactly opposite, but separated by two more rows on one side than on the other. (See fig. 1, E. The pedicelled spikelets of a pair of yoked alicoles are marked *a*. It will be seen that they are separated on one side by six rows and on the other by eight.)

The persistence with which ears of maize maintain an even number of rows is therefore more wonderful than has been supposed, for it can not be fully accounted for by the fact that the spikelets are born in pairs. It must, in addition, be recognized that when a pedicelled spikelet of one alicole is suppressed there is a simultaneous suppression of the pedicelled spikelet in another alicole. The further evidence afforded by Euchlaena hybrids is that the two alicoles are the members of a yoked pair which though standing on opposite sides of the ear, have not lost their identity as a structural and developmental unit.

SUMMARY

Before the pistillate inflorescences of maize and Euchlaena could be compared in detail it was found necessary to recognize as a morphological unit the organs borne by a single metamer of the rachis. This unit, whether staminate or pistillate, whether composed of one or more spikelets, has been called an alicole.

The stages between a Euchlaena spike and a maize ear as they appear in hybrids between the two genera may be summarized as follows:

(1) The suppressed pedicelled spikelet in each alicole reappears. (2) The alicoles become more crowded and their number is increased. (3) The alicoles associate themselves in pairs or yokes. (4) The axis twists, increasing the rows of alicoles.

The order in which these changes occur is by no means fixed, but taken together they comprise all the changes necessary in deriving the maize ear from the Euchlaena spike.

In this series of intermediate stages nothing was observed that affords support for either the fasciation or "reduced branch" theory of ear formation. There is also evidence from the maize ear itself that the association of alicoles into pairs is more fundamental than the linear arrangement.

In all the hybrids between maize and Euchlaena that have been observed there has appeared no suggestion of either pod corn or *Zea ramosa*. Since it can scarcely be doubted that the peculiar characteristics of both of these mutations represent the reappearance of ancestral characters common to the Andropogoneae, it would seem that in crossing maize and Euchlaena, and thus calling forth a series of intermediate forms, we are not returning to the point in the ancestry of maize where it became differentiated from the Andropogoneae.

Furthermore, if the stages shown in the hybrid plants were to be taken as indicating the path of evolution of the ear, it would be necessary to assume that the central spike of the staminate inflorescence or tassel had evolved separately and along different lines. The close homology between the ear and the central spike of the tassel makes such an assumption unreasonable.

In the present article emphasis has been placed on the shortening and twisting of the axis of a single spike as a possible method of deriving a structure like the maize ear from the inflorescence of Euchlaena. This has been done, not because the method is believed to represent the most probable course of evolution, but because the present discussion has been restricted to the evidence afforded by hybrids of maize and Euchlaena, which seems to require such an interpretation.

Facts of other kinds are more easliy interpreted by the theories of fasciation and reduction of branches, but there are also facts that do not seem to accord with any of the theories yet proposed. Until the apparently contradictory evidence can be reconciled, it seems best to keep the several possibilities in mind and await additional evidence before attempting a complete interpretation.

PLATE 16

Intermediate stages between a simple spike of the pistillate inflorescence of Euchlaena and an ear of maize:

A.—Spike of pure Florida teosinte.

B.—Spike with slightly shortened axis.

C.—A still more compact spike with an increased number of seeds. A–C have single spikelets and separate two-ranked alicoles.

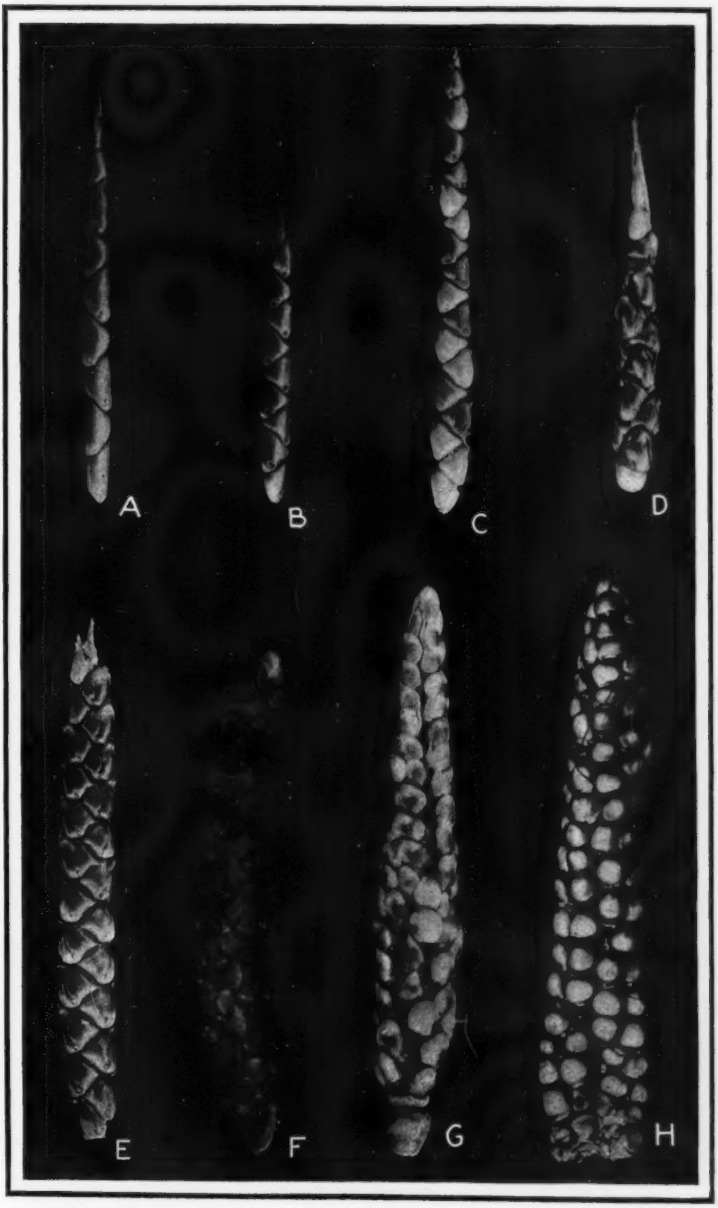
D.—Spike with single spikelets and yoked alicoles, irregularly four-rowed.

E.—Compact spike with two-ranked separate alicoles and single spikelets.

F.—Spike with paired spikelets and four ranks of yoked alicoles.

G.—Transition stage between four-rowed and eight-rowed ear.

H.—Ear of maize with eight rather poorly defined rows of seeds.


A
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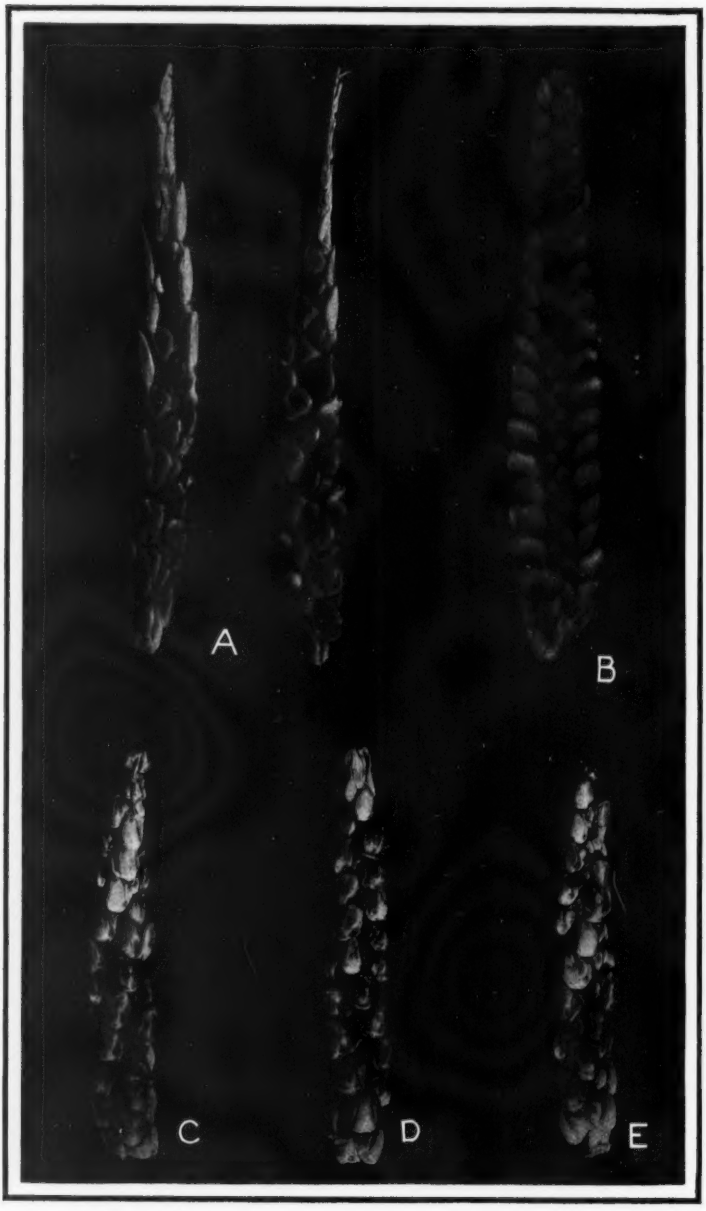

A
B
C
D
E

PLATE 17

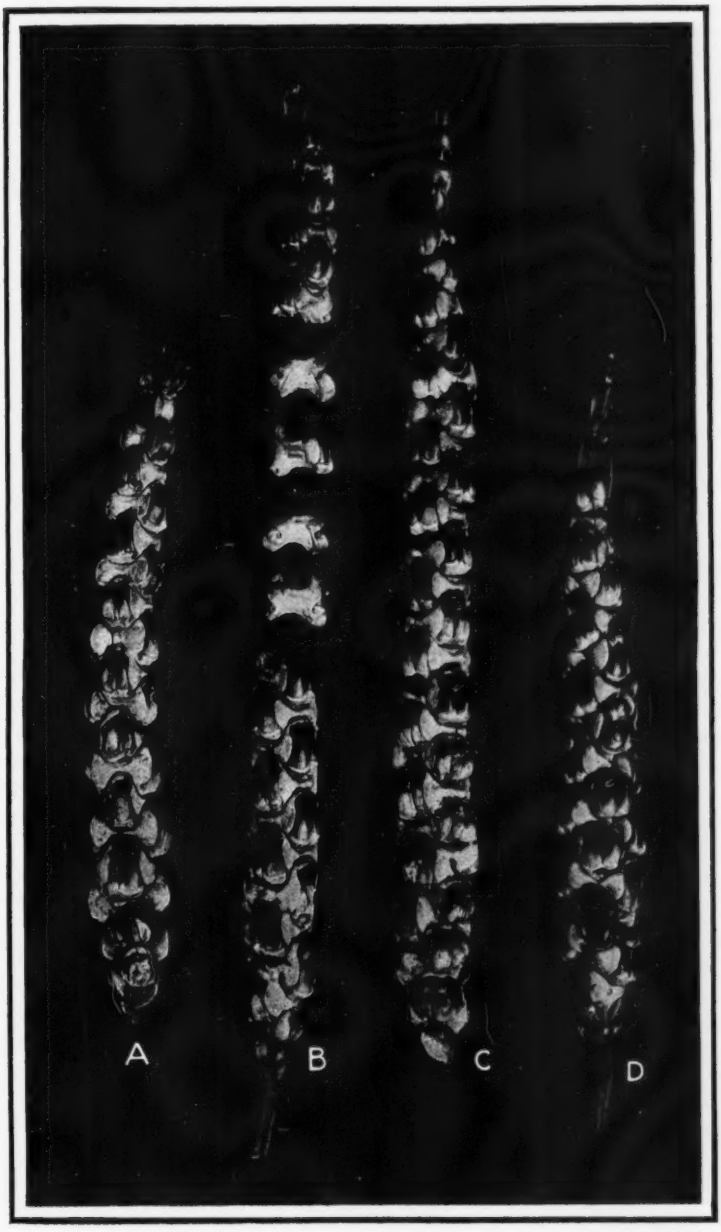
Pistillate inflorescences of hybrid between Euchlaena and maize:
A.—Showing pedicelled staminate spikelets with sessile pistillate spikelets.
B.—Closely compacted inflorescense with two rows of alicoles and four rows of seeds.
C–E.—Spirally twisted inflorescences, with three rows of alicoles.

PLATE 18

Pistillate inflorescences of hybrid between Euchlaena and maize, showing yoked alicoles:

A–C.—The alicoles are in four rows corresponding to an eight-rowed ear.

D.—The alicoles are in five rows, corresponding to a ten-rowed ear.


A
B
C
D